

Review

Molecular and Genetic Bases of Fruit Firmness Variation in Blueberry—A Review

Francesco Cappai^{1co}, Juliana Benevenuto^{2co}, Luís Felipe V. Ferrão^{3co} and Patricio Munoz^{4,*}

¹ Blueberry Breeding and Genomics Laboratory, Horticultural Sciences Department, University of Florida, Gainesville, FL, USA; francesco.cappai@ufl.edu

² Blueberry Breeding and Genomics Laboratory, Horticultural Sciences Department, University of Florida, Gainesville, FL, USA; jbenevenuto@ufl.edu

³ Blueberry Breeding and Genomics Laboratory, Horticultural Sciences Department, University of Florida, Gainesville, FL, USA; lferrao@ufl.edu

⁴ Blueberry Breeding and Genomics Laboratory, Horticultural Sciences Department, University of Florida, Gainesville, FL, USA; p.munoz@ufl.edu

^{co} These authors contributed equally to this work

* Correspondence: p.munoz@ufl.edu; Tel.: +1-352-273-4837

Abstract: Blueberry (*Vaccinium spp.*) has been recognized worldwide as a valuable source of health-promoting compounds, becoming a crop with some of the fastest rising consumer demand trends. Fruit firmness is a key target for blueberry breeding as it directly affects fruit quality, consumer preference, transportability, shelf life, and the ability of cultivars to be machine harvested. Fruit softening naturally occurs during berry development, maturation, and postharvest ripening. However, some genotypes are better at retaining firmness than others, and some are crispy, which is a putatively extra-firmness phenotype that provides a distinct eating experience. In this review, we summarized important studies addressing the firmness trait in blueberry, focusing on physiological and molecular changes affecting this trait at the onset of ripening and also the genetic basis of firmness variation across individuals. New insights into these topics were also achieved by using previously available data and historical records from the blueberry breeding program at the University of Florida. The complex quantitative nature of firmness in an autopolyploid species such as blueberry imposes additional challenges for the implementation of molecular techniques in breeding. However, we highlighted some recent genomics-based studies and the potential of a QTL mapping analysis and genome editing protocols such as CRISPR to further assist and accelerate the breeding process for this important trait.

Keywords: firmness; *Vaccinium*; ripening; cell wall; crispy; quantitative genetics; breeding; molecular markers; genome editing

1. Introduction

Blueberry has been recognized worldwide for its health benefits due to its high content and wide diversity of polyphenolic compounds [1,2]. Polyphenolic compounds, especially anthocyanins, have been shown to have anti-oxidant, anti-inflammatory, anti-proliferative, anti-obesity, and neuroprotective properties [3]. Such health-related awareness has been driving an increase in demand for blueberries, which became one of the crops with the highest production trends. From 1996 to 2016, worldwide production has grown by 72,11% [4]. Currently, the United States of America is the largest producer, being responsible for around 48% of the world's production in 2016 [4].

Most of the blueberry production is destined for the fresh market sector, which requires high-quality berries and postharvest longevity [5]. In order to maintain the fresh market standards,

blueberries are usually hand-picked. However, hand-harvest labor accounts for 50% (or even more) of the production costs and raises concerns about its long-term availability [5–7]. In addition, fresh fruit handling by ill workers has been linked to foodborne illnesses in consumers [8]. Mechanical harvesting can mitigate the need of hand-harvest labor, decrease production costs, and foster further expansion of this healthy fruit. Wide adoption of machine harvesting for the fresh market is, however, currently limited to only a few commercial blueberry cultivars. Fruit firmness is one of the main determinant traits required to withstand the physical impacts during machine harvesting [6,7,9–11].

Blueberries are shipped to long-distance markets all over the world and softening and bruising fruits are among the most common defects causing shipment rejections [10,12]. Hence, firm berries are also critical for transportability and shelf-life longevity [10,11]. Moreover, a firm texture is one of the consumers' most appreciated features, being associated with the general concept of fruit freshness and quality [13–15]. Taken all together, fruit firmness is a key target for blueberry breeding as it benefits all stakeholders.

Firmness naturally declines during berry development, maturation, and postharvest ripening [12,16,17]. Understanding the biological mechanisms underlying fruit softening is essential to manipulate it without affecting other desirable aspects of ripening, such as color, flavor, aroma, or nutritional value [18]. Despite the natural softening during ripening, some plant genotypes appeared best at retaining firmness by the merit of having high initial firmness values [9]. Intraspecific (*V. corymbosum*) and interspecific (wild relatives) phenotypic variation has been reported, constituting breeding resources for firmness improvement [6,19]. In addition to firmness, a potentially separate phenomenon has been identified in blueberry: fruit crispiness. Crispy fruits are firmer and have higher bursting energy than standard fruits. Hence, crispiness is a distinct textural attribute that is also of high interest for the blueberry industry.

In this review, we presented recent advances in blueberry research regarding firmness changes during berry development and ripening. We summarized the extent of phenotypic variability of the firmness trait described in blueberry and its genetic parameters. New insights into these topics were achieved by using previously available data and historical records from the blueberry breeding program at the University of Florida. We also emphasized the first study attempting to implement marker-assisted selection (MAS) for this trait, through genome-wide association studies (GWAS). Finally, we discussed perspectives to further implement QTL mapping and genome editing technologies such as CRISPR in blueberry.

2. Physiological, cellular, and molecular changes affecting fruit firmness

2.1. Fruit anatomy and growth during ripening

Blueberries, as many berries of the *Vaccinium* genus, are false berries, because they are berry-like, but develop into a fruit without a stone from a single fertilized superior ovary [20]. Anatomically, the blueberry fruit develops from an inferior ovary. The endocarp is composed of five carpels with ten locules and five lignified placentae to which around 10–65 seeds are attached. The endocarp is surrounded by the mesocarp, which is composed of parenchyma cells along with rings of vascular bundles, with some stone cells unevenly distributed [15,21]. The epicarp originates from the flower calyx. Delimited by a ring of vascular bundles, the hypodermal layer contains the anthocyanin pigmentation. Above this, there is a single layer of epidermis without stomates. The epidermis is covered by a cuticle and an epicuticular waxy bloom that overshadows the purple-black skin of blueberry fruits, creating the characteristic light-blue color [22].

Berry growth commonly exhibits a double sigmoid growth curve, with a lag stage between two phases of active growth [23,24]. The first growth stage occurs after syngamy and is characterized by a rapid cell division, leading to a rapid expansion of the pericarp. In the second stage (lag stage), embryo and endosperm tissues mature, while the development of the pericarp is retarded with no evident increase in berry size and no changes in color. In the third stage, a second

rapid pericarp development takes place due to cell enlargement until the berry is fully ripe. Changes in size are accompanied by changes in color (from green, pink, to blue), and biochemical changes such as increases in pH, sugar composition, soluble solid content, volatiles, and texture. The fruit development and ripening process usually takes 45-90 days, depending on the cultivar and external factors [25].

2.2. Ripening-associated physiological changes

Physiological changes associated with ripening are a major determinant of berry firmness, as the fruit softens as it matures. The ripening process is coordinated by a complex network of endogenous hormones. For many fleshy fruit plant species, the phytohormone ethylene is the main ripening agent [26]. Climacteric fruits show a concomitant increase in respiration and ethylene biosynthesis upon initiation of ripening [27]. Ethylene perception and signal transduction can affect fruit color, sugar and acid content, and firmness [28]. This process is however not linear: it varies over time and the abovementioned ripening changes can be disjointed [28,29]. Currently, there is no consensus on whether blueberry is a climacteric fruit or not. Some studies suggested that blueberry is indeed a climacteric fruit [30,31], with also increased anthocyanin accumulation upon ethylene application in some cultivars [32]. Chiabrando and Giacalone (2011) [33] proposed that 1-methylcyclopropene (1-MCP), a compound that hinders cellular ethylene perception, has the potential for controlling ripening in blueberry. Fruits from the cultivar "LateBlue" showed reduced post-harvest weight loss and lower total soluble solid content after 1-MCP treatment, indicating a slower ripening effect [33]. In line with these findings, Wang et al. (2018) [34] also showed that an ethylene absorbent treatment prevented ethylene production, inhibited cell wall degrading enzymes, and reduced softening of blueberry fruit. However, conversely, other studies suggested that 1-MCP application has no inhibition effect on ripening of post-harvested blueberries [35,36], and might decrease fruit firmness [37,38].

In non-climacteric fruits, where no burst in ethylene production is observed, abscisic acid (ABA) seems to have a stronger role during fruit ripening [27]. However, similarly to what has been reported for ethylene, studies addressing the effects of ABA in blueberry fruit firmness also showed contradictory results. Sun et al. (2013) [39] reported that ABA application was able to promote fruit softening; whereas Buran et al. (2012) [40] observed the opposite phenomenon.

As of now, the role of ethylene and ABA phytohormones in blueberry fruit ripening as well as the question if blueberry is a climacteric or non-climacteric fruit remains unclear. However, it is noteworthy how multiple studies have reported different effects of ethylene, as inhibitor or promoter of fruit ripening, and also cultivar-specific responses, suggesting the need for more studies in this field and that distinct mechanisms might exist among blueberry genotypes.

2.3. Molecular and architectural changes in plant cell during fruit ripening

Ripening is also accompanied by compositional and architectural changes in the plant cell, mainly at the primary cell wall and middle lamella through the action of carbohydrate active enzymes, ultimately affecting fruit firmness [41]. Plant cell wall is a complex matrix of polysaccharides, mainly composed of pectin, hemicellulose, and cellulose. The cellulose microfibrils are cross-linked through hydrogen bonds with hemicellulose, thereby forming a complex network that provides tensile strength to the primary cell wall [42]. The pectin matrix is interwoven with the cellulose-hemicellulose network in the primary cell wall and also composes the middle lamella, being a major physical mediator of cell adhesion and separation [43].

Noncellulosic sugars have been implied in regulating firmness as they provide physical support by connecting at cellulose myofibrils in cell walls [44]. In particular, depolymerization and solubilization of hemicellulose and pectin are the processes generally associated with cell wall disassembly and fruit softening during ripening [41,45–48]. In blueberry, hemicellulosic polymers undergo a significant depolymerization and a moderate solubilization throughout all five stages of ripeness analyzed, indicating that hemicellulose modification might be the main cell wall alteration during blueberry ripening [49]. The pectin matrix also shows increased solubilization mostly at the initial and intermediate stages of ripening and little reduction in polymer size occurred [49]. Pectin solubilization was also observed during blueberry post-harvest storage [17]. Interestingly, calcium application has been reported to enhance firmness and post-harvest quality of blueberries [50,51]. Angeletti et al. (2010) [51] speculated that the increase in calcium content might decrease pectin solubilization, while not affecting hemicellulose in blueberry. In many ripe fruits, most of the cell-to-cell adhesion is conferred by calcium-pectate cross-links in the middle lamella, producing a semirigid gel that increases cell wall stiffness and hinders cell wall disassembly [41,43,52,53]. In general, the role of calcium in cell wall solidity and post-harvest quality is well established in a number of crops, such as apple [54–56], grape [57–59], strawberry [60–62], and seems to be relevant also in blueberry.

The degradation of cell wall polysaccharides depends on the action of enzymes with distinct spectra of activities. To further understand the role of Carbohydrate-Active Enzymes (CAZymes) during blueberry ripening, we retrieved the putative encoding genes by sequence similarity of the predicted blueberry proteome with the CAZy database (<http://www.cazy.org/>) and the transcriptional profile of five stages of berry fruit development (pad, cup, green, pink, and ripe fruits) from Gupta et al. (2015) [63]. Distinct patterns of expression were observed for the genes into the six classes of CAZymes throughout the fruit development process (Figure 1). Up-regulated CAZymes encoding genes in pink and ripe fruits in relation to early stages are highlighted as they can be involved in cell wall disassembly, allowing cell expansion and fruit growth, but later cause fruit softening.

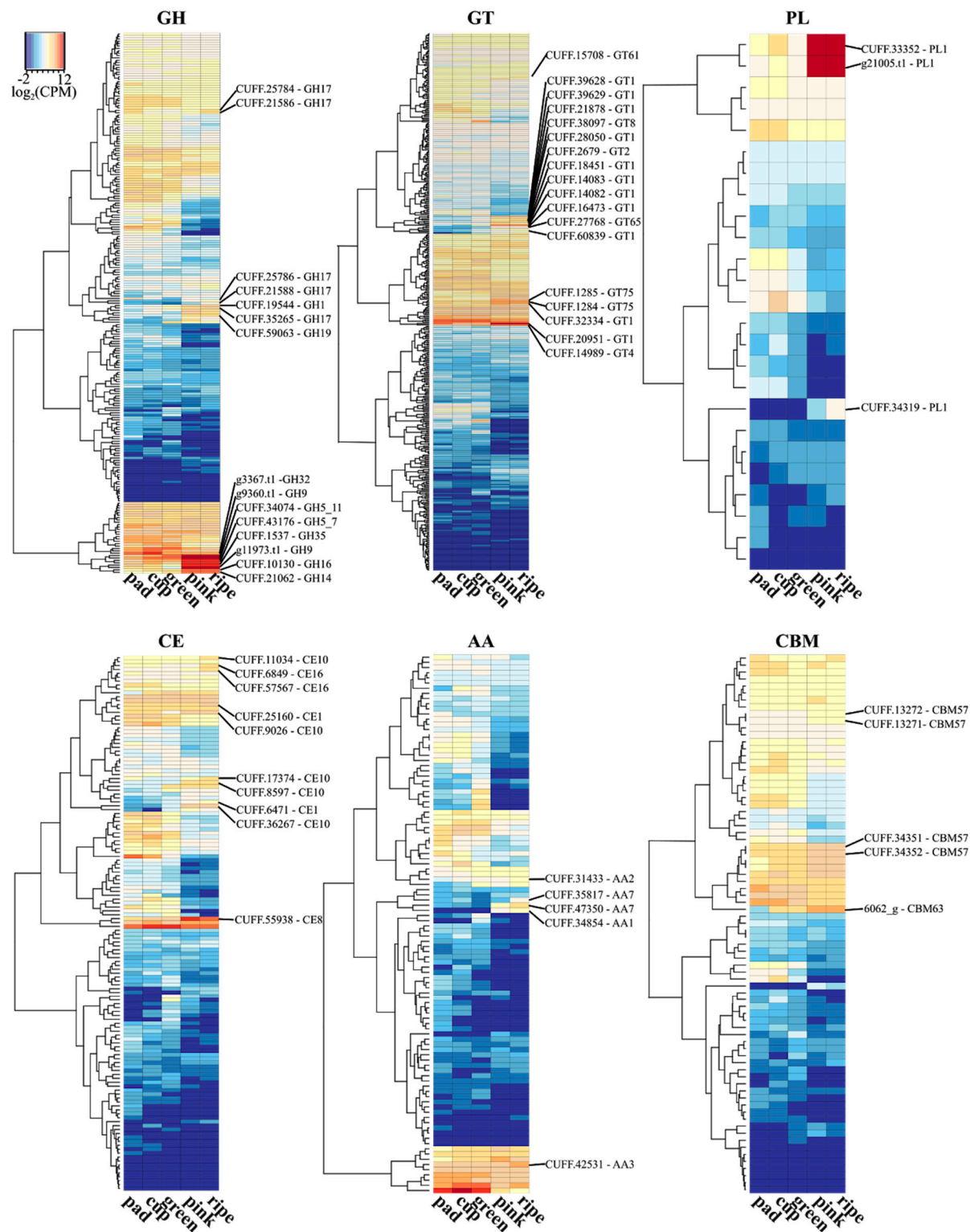


Figure 1. RNA-seq transcriptional profile of CAZymes during five stages of berry fruit development and ripening. The predicted blueberry proteome was screened for CAZymes [64] using Hmmscan from the HMMER v3.1b2 package (<http://hmmer.org/>) and the dbCAN HMM profile database [65]. The hmmscan-parser script provided by dbCAN was used to select significant matches. CPM (Counts Per Million mapped reads) values from five developmental berry stages of a RNA-seq experiment were retrieved from Gupta et al. (2015) [63] from the bitbucket repository (<https://bitbucket.org/lorainelab/blueberrygenome>). The CPM mean value of each gene was calculated from three replicates of each stage available. The log₂-transformed CPM mean values were used to plot the gene expression of CAZymes across the five stages. The R package "gplots" with "heatmap.2" function was used to generate the heatmaps

using the parameter "dendrogram = c("row")" to cluster genes according to hierarchical clusters. Classes and modules of CAZymes are represented by GH (glycoside hydrolases), GT (glycosyl transferases), PL (polysaccharide lyases), CE (carbohydrate esterases), AA (auxiliary activities), and CBM (carbohydrate-binding modules) (For more details, see: <http://www.cazy.org/>). Genes up-regulated at pink and/or ripe stages in relation to early stages are highlighted and the family number reported.

Glycoside hydrolases (GHs) catalyze the hydrolysis of glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Among the up-regulated GHs families, we found five beta-glucosidases (GH family 17) blueberry encoding genes (Figure 1). GH 17 family members have been associated with cell wall degradation and remodeling, hydrolysis of phytohormones (such as ABA), pathogen resistance, and in aromatic acid biosynthesis pathways [66]. In banana, more than two-fold enhanced expression of genes in the GH 17 family were detected in the ripe fruits compared to unripe ones [66]. Others interesting GH enzymes detected were endo-1,4- β -glucanases (GH7, GH9), exo-1,4- β -glucanases (GH1), endo-xyloglucan transferase (GH16), and endo-1,4- β -xylanase (GH11) that probably act on hemicellulose depolymerization or hydrolyzing the crosslinks between microfibrils, thereby disassembling the cellulose-xyloglucan network [67,68]. Exo- β -galactosidases from GH family 35 has also been associated with depolymerization of pectins. These enzymes can cause cell wall loosening during growth, and cell wall degradation during ripening and senescence [66,69]. Up-regulated invertase (GH32) and amylase (GH14) generate reducing sugar monomers, which may be responsible for the increased sweetness during fruit ripening [70]. In blueberry, cold storage was shown to maintain firmness probably due to the reduced activities of such enzymes at lower temperatures [36].

Polysaccharide Lyases (PLs) cleave uronic acid-containing polysaccharide chains. Three pectate lyase (PL1 family) genes were up-regulated during blueberry ripening (Figure 1). PL1 enzymes degrades de-esterified pectin in the primary wall. In tomato, the silencing of a PL-encoding gene inhibited pectin solubilization and depolymerization, maintaining the firmness without affecting other aspects of ripening [18,71]. In line with this, a gene in the carbohydrate esterase (CEs) class, encoding a pectin methylesterase (CE8), was also up-regulated in blueberry (Figure 1). CE8 enzymes alter the pectin structure by catalyzing the demethyl esterification of pectin, which then becomes cleavable by pectate lyases [18]. Glycosyltransferases (GTs), which catalyze the transfer of sugar moieties forming glycosidic bonds, have been associated with cell wall polysaccharide synthesis (GT2, GT8, GT61, GT65, GT75), sucrose synthesis (GT4), and anthocyanin modification and formation of glycosylated volatile compounds during fruit development and ripening [72,73]. Enzymes with auxiliary activities (AAs) that act in conjunction with CAZymes and genes with a carbohydrate-binding module were also found up-regulated at pink and blue stages of blueberry ripening (Figure 1).

Besides changes in cell wall composition and disassembly, reduction in turgor pressure during ripening can also cause shrinkage and loss of firmness. Reduction in the turgor is likely due to the accumulation of osmotic solutes in the apoplast and to water loss by the fruit [41] and has been reported in stored blueberries [74]. Recent studies had also shown the roles of the stem scar size/transpiration and cuticular wax composition and thickness in water loss and maintenance of the post-harvest firmness in blueberry [22,75,76]. Among the blueberry fruit cuticular triterpenoid composition, the ursolic acid content at harvest was positively correlated to weight loss and softening after storage, offering an interesting target for further studies [76]. The importance of containing water loss is also supported by the fact that coating fruits with oily films, which create a semi-permeable barrier around the fruit, prevented firmness decay [77,78].

The findings reported herein offer some insights into the molecular bases of fruit softening during ripening and postharvest storage of blueberries. Further functional validation of the differentially expressed CAZymes can provide good candidates for the implementation of genome editing tools aiming at firmness maintenance.

2.4. Fruit tissue and cellular differences underlying firmness variation

Several components of fruit tissues have been reported to contribute to firmness and texture variation across genotypes, including cell type, size, shape, packing, cell-to-cell adhesion, extracellular space, and cell wall thickness [15,79,80]. In blueberry, differences regarding the number and organization of lignified cells with thick secondary cell walls, such as stone cells in the mesocarp, have been hypothesized to strengthen the surrounding flesh tissue and contribute to fruit firmness variation among genotypes [80,81]. Sensorial studies of genotypes ranging from soft to crisp suggested that the crisp texture may be related to the blueberry skin toughness rather than the flesh [82]. Histological analyses of cell type, area, and structure of the outermost cell layers of genotypes varying in textural attributes showed that crispy genotypes had a smaller average cell area compared with standard-textured genotypes in mature fruits and no difference in the frequency of stone cells in the layers beneath the epidermis was detected [15].

3. Genetics and breeding of blueberry firmness

3.1 Measuring firmness

Fruit firmness has long been evaluated by chewing texture, which is a subjective and qualitative score ranging from “soft” to “firm” [83]. More recently, automated equipment allowed the objective and quantitative evaluation of fruit firmness. FirmTech instruments (FirmTech I and II, Bioworks, Wamego, KS) are the most commonly used for blueberry and firmness is a measure of the compression force (g) required to deflect the surface of the fruit one millimeter. Texture Analyzer instrument (Texture Technologies Corporation, Scardale, NY) has also been increasingly used and might give more precise results than FirmTech, especially when trying to dissect skin and pulp firmness. However, these equipment present differences in probe sizes and shapes, making the standardization challenging [82,84–87]. Hence, in this review, we only included measurements obtained with FirmTech instruments as more data were available [6,14,37,80,88–123].

Regarding the firmness trait evaluation, an issue that has not been addressed yet is how many berries should be sampled to accurately represent a genotype. Each study has used a distinct number of samples, ranging from five to hundreds of berries. Assuming that sample size is an important element for research design and validation, we assessed how the mean firmness varied across different sample sizes considering four cultivars (Figure 2). We collected 200 berries from each genotype and performed 1,000 resamplings considering 12 different sample sizes. The mean and variance values computed for the 200 berries were considered our “true” population parameters. As expected, small sample sizes resulted in higher variance in the mean firmness values of a genotype than large sample sizes (Figure 2a). The fraction of the confidence intervals (CI=90%) that encompassed the “true” population mean ranged across the sample sizes for all cultivars (Figure 2b) and, the smaller the sample size, the more values out of the CI were generated. For a sample size of five berries and for all cultivars, we observed that around 25% of the estimates did not lie in the confidence interval of 90%, which means that in a breeding population composed of 1000 individuals around of 250 measures of firmness would be inaccurate, hence affecting downstream analyses.

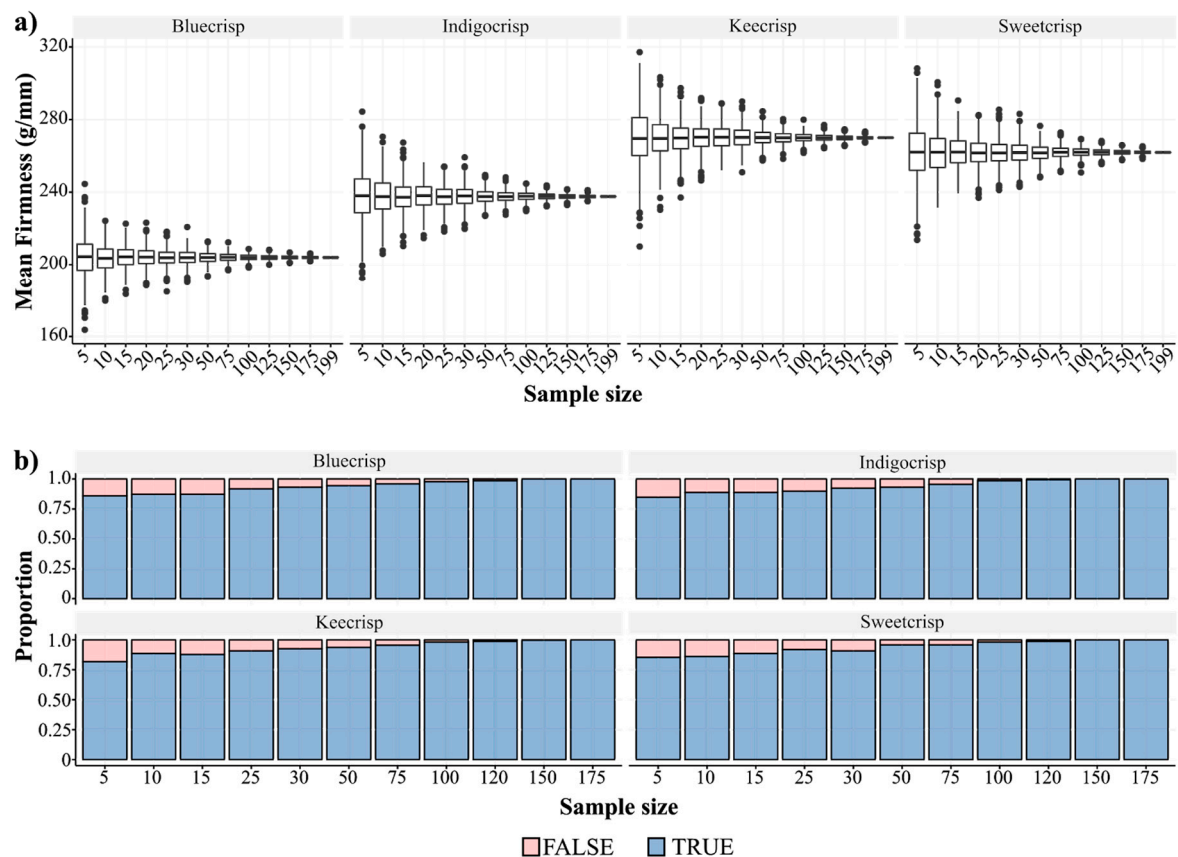


Figure 2. Number of berry samples to estimate the firmness value of a genotype. **a)** Boxplot of the mean firmness values considering different sample sizes for four SHB cultivars. Briefly, 200 mature berries of each cultivar were collected and measured for firmness using the FirmTech II device (BioWorks Inc.). To describe the effect of the sample size on the mean value, 1000 resamplings were carried out considering 12 different sample sizes (5–199) and the mean firmness value was computed at each sampling round. **b)** Fraction of the confidence intervals that encompassed the “true” mean parameter, computed assuming that 200 berries are enough to well-represent a genotype, across different sample sizes and four different cultivars. We performed the same resampling process but recording the parameters that laid in a confidence level of 90%.

3.1. Phenotypic variation and breeding improvement in fruit firmness

Heritable phenotypic variation is critical for breeding selection to be effective. Enough phenotypic variation for firmness has been observed within breeding populations, among blueberry cultivars, and wild species [6,19,124]. Herein, we performed a survey of firmness values for a wide range of cultivars, representing the main cultivated blueberry types. Cultivated blueberry belongs to section *Cyanococcus* of the genus *Vaccinium* into the Ericaceae Family and comprises distinct species and hybrids with specific ploidy, plant architecture, and chilling hour requirements. The main blueberry type used for commercial production is a tetraploid highbush ($2n=4X=48$), with *Vaccinium corymbosum* L. as the primary species in its genetic background. Highbush blueberries are further classified according to their chilling requirements as Northern Highbush Blueberry (NHB) and Southern Highbush Blueberry (SHB) [125,126]. NHB is native to eastern North America and is the most widely planted type in temperate climates (600–1,200 hours of chilling between 0 to 7° C). SHB was originally developed by the introgression of an evergreen Florida native species (*Vaccinium darrowii* Camp) into the NHB background, leading to reduced chilling requirements (100 to 600 hours) and, therefore, being adapted to warmer climates such as southern US [127–129]. Rabbiteye blueberry is another commercially important species, hexaploid *Vaccinium ashei* Reade, that tolerates a range of soil and warm climatic conditions and are also

294 planted in the southern US. Lowbush blueberry is another tetraploid species (*Vaccinium*
295 *angustifolium* Aiton), whose wild fruits are harvested commercially in New England. The cultivated
296 half-high blueberry (HH) was originated from a cross between NHB and lowbush and also requires
297 lower temperatures to flower.

298 In this survey, we collected berry firmness data from scientific papers that used the FirmTech
299 instrument, except for those reporting experimental treatments such as insect damage, extreme
300 growth conditions, etc. (see Supplementary Material for more details). The mean firmness value of
301 each cultivar was plotted according to their respective release year (Figure 3). High diversity of
302 firmness values can be observed between and within blueberry types (Figure 3a). As the NHB
303 breeding began earlier (1906), more NHB cultivars are displayed. The first released NHB cultivars,
304 "Harding" and "Rubel", were actually selected from the wild. "Pioneer" was the first cultivar
305 released after breeding efforts in 1920 [130]. SHB breeding programs are more recent and the first
306 cultivar "Sharpblue" was released in 1975 [131]. The NHB cultivar "Herbert" presented the lowest
307 firmness value, while the SHB cultivar "Sweetcrisp" presented the highest firmness value among all
308 blueberry types (Table 1).

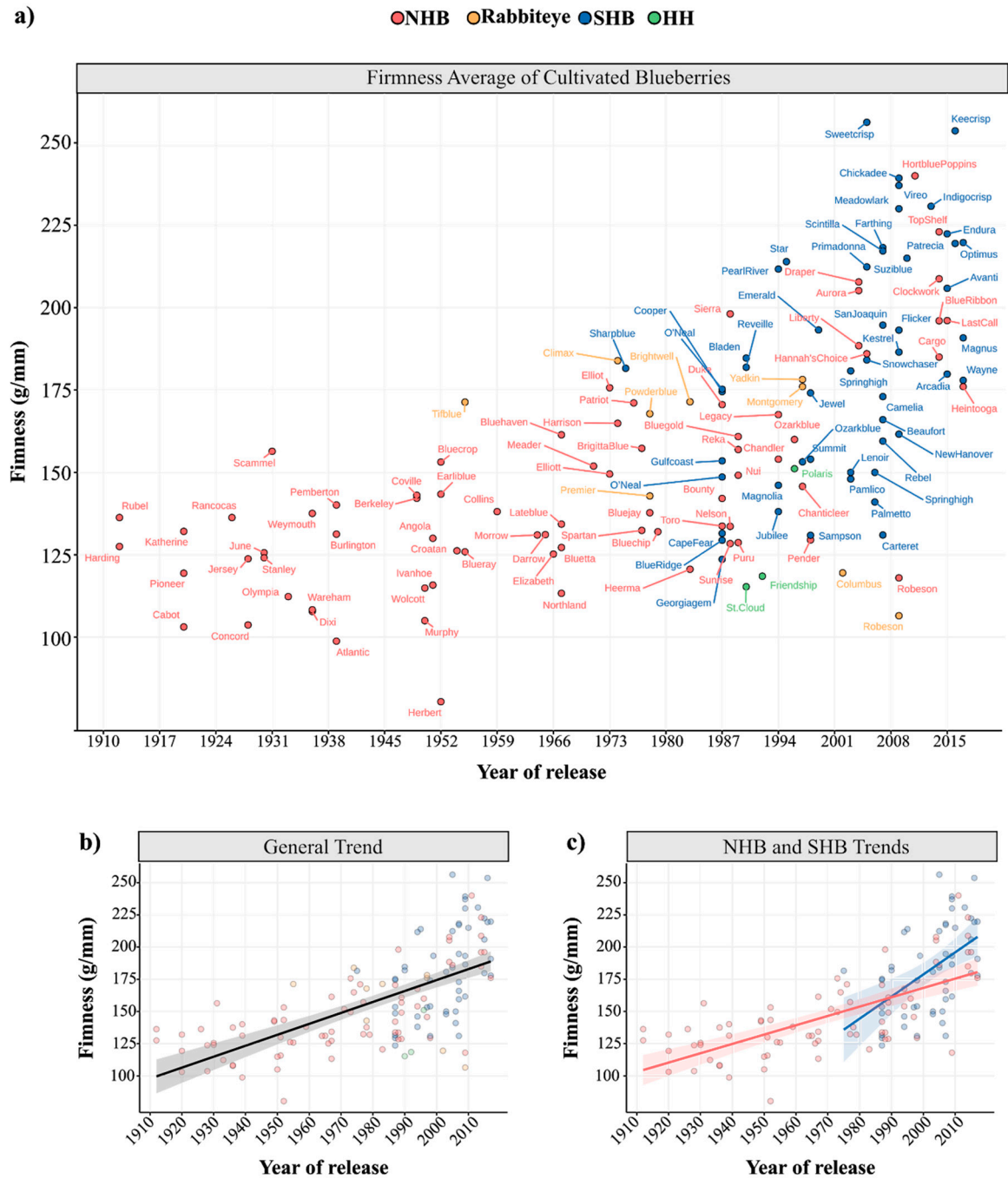


Figure 3: **a)** Blueberry cultivar firmness values by year of release. Plot was created using historical data recorded by the blueberry breeding program at the University of Florida and data from previous blueberry publications (for details, see Supplementary Material). **b)** Linear regression on the firmness values as a function of the year of cultivar release with a slope of 0.84. **c)** Linear regression on the firmness values as a function of the year of cultivar release for NHB (slope of 0.72) and SHB (slope of 1.71), separately.

Table 1: Summary of firmness measurements for distinct types of cultivated blueberry. For more detailed information, see Supplementary Material.

Type*	n	Mean	St.Dev	Maximum	Minimum
SHB	50	183	35.18	256 (Sweetcrisp)	124 (Georgiagem)
HH	3	128	19.79	151(Polaris)	115 (St.Cloud)
NHB	74	145	31.22	240 (Hortblue Poppins)	80 (Hebert)
Rabbiteye	9	157	27.87	184 (Climax)	106 (Robeson)

* Southern Highbush (SHB), Northern Highbush (NHB), Half-high (HH), and Rabbiteye cultivars.

The positive regression slope in Figures 3b showed a general trend towards firmness improvement throughout the years, i.e., on average, modern cultivars are firmer than first-released genotypes. This general trend is expected, as firmness is a key target trait in blueberry breeding programs and also suggested that significant genetic gain has been achieved. In Figure 3c, we can also observe a trend towards higher firmness values of SHB in relation to NHB, although SHB also exhibit slighter higher variation (Table 1). Some studies suggested that cultivars with a higher percentage of *V. darrowii* (evergreen blueberry) and *V. ashei* (rabbiteye) ancestry often possessed higher firmness values, which would be the case of SHB; while cultivars with *V. angustifolium* (lowbush) ancestry presented softer fruits than the average, as also observed for HH blueberries in Figure 3a [6,83,124]. However, such conclusions can be misleading since the firmness values retrieved herein were collected under distinct experimental conditions and locations, using varying numbers of berries and equipment versions (see Supplementary Material).

It is also noteworthy that cultivars considered crispy, such as “Sweetcrisp” (SHB), “Keecrisp” (SHB), “Indigocrisp” (SHB), “Hortblue Poppins” (NHB), showed the highest firmness values (Figure 3a). Blaker et al. (2014) [91] had also found a correlation among compression and bioyield force measures with sensory scores for bursting energy, flesh firmness, and skin toughness, distinguishing crisp from standard-texture SHB genotypes. We should also mention that in food science jargon crunchiness and crispness are two different phenomena based on sensory, acoustic and vibrational cues [132]. However, no study has formally addressed whether blueberry cultivars are crispy or crunchy.

3.3. Quantitative genetics of firmness trait in SHB blueberry breeding

Blueberry breeding programs have relied on pedigree information and cross-pollination breeding methods to increase the mean phenotypic performance in selected populations [126]. Phenotypic recurrent selection has been used as a primary breeding strategy in blueberry, where elite parents are selected at each generation for intercrossing, the progenies are evaluated over the course of multiple years in the field, and a cultivar consists of a superior individual cloned by cutting propagation. During this process, quantitative genetics constitute the fundamental basis to guide breeding efforts. Many agronomically important traits, including firmness, display a continuous phenotypic variation in a given population, likely governed by the joint action of numerous genes and environmental factors. The continuous distribution of such complex traits resulted in phenotypes that do not show simple Mendelian inheritance, requiring a quantitative genetics framework of analyses. Quantitative models dissect the observed phenotypic value of an

individual into genetic and environmental components in order to estimate the heritable and non-heritable portions of the variation.

Quantitative genetic studies in blueberry have focused on three main challenges: I) identify the elite genotypes to be used as parents in future crosses [19,133–136]; II) estimate genetic parameters such as heritability, phenotypic and genetic correlations, and predict the expected change in a trait in response to selection – the breeder’s equation [19,133]; and III) estimate the genotype-by-environment (G×E) interaction [13,19,137,138]. Genetics studies in blueberry have been reported for distinct traits; however, to our knowledge, only Cellon et al. (2018) [19] have investigated the firmness trait. In order to guide the selection of firmer genotypes and estimate genetic parameters, Cellon et al. (2018) [19] found moderate-to-high values of heritability for this trait with large variability across the years (0.43 in 2014 and 0.70 in 2015). The presence of variability across the years indicated genotype-by-year interaction, while the magnitude of the heritability values suggested potentially rapid gains in response to selection.

Phenotypic expression of quantitative traits is influenced by environmental conditions, leading to variations across locations and years that, ultimately, impact the ranking of the genotypes. Despite its relevance, there are few studies addressing G×E for the firmness trait in the blueberry literature. Motivated by this, we performed an initial assessment of G×E interaction in SHB using the data set recorded by the breeding program at the University of Florida for six cultivars, planted in four locations, from 2011 to 2017 (Figure 4a). Based on an analysis of variance (ANOVA), the G×E interaction effect was statistically significant ($P<0.05$). The interaction plot suggests that the crispy cultivars, “Indigocrisp” and “Sweetcrisp”, are more stable across the environments than the non-crispy ones (Figure 4b). This preliminary screening suggests that G×E interaction impacts fruit firmness. From a theoretical point of view, ignoring the heterogeneity caused by different environments can bias the predictions of breeding values and negatively affect the estimates of genetic variances. For practical purposes, such effect directly affects the selection of elite materials and decreases genetic gains along breeding cycles [139].

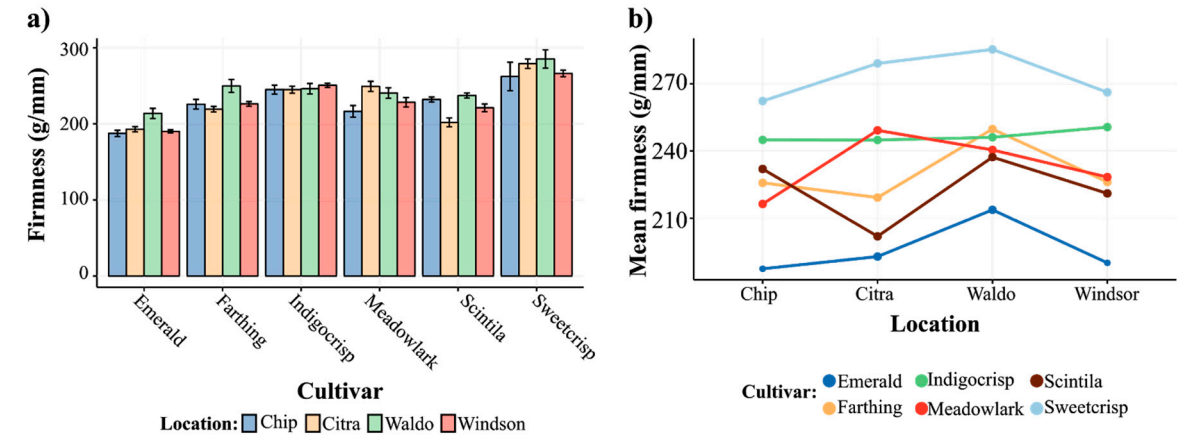


Figure 4. Genotype-by-environment interaction in SHB using the historical data recorded by the breeding program at the University of Florida. **a)** Average of firmness for six cultivars, evaluated across four locations, during seven years. **b)** Interaction plot showing the changes in firmness profile for six cultivars across four locations.

3.4 Marker-assisted selection for fruit firmness

Genomics-based strategies are increasingly being used to assist crop improvement. Advances in next-generation sequencing (NGS) and genotyping technologies have enabled high-throughput and relative low-cost identification of single-nucleotide polymorphisms (SNPs). By using thousands of markers spread throughout the genome, genome-wide association studies (GWAS) and genomic selection (GS) methods are becoming popular in many crop species [140]. Despite the potential of these approaches to accelerate the breeding programs, there are remarkably few studies aiming to implement marker-assisted selection (MAS) in blueberry.

A recent GWAS in SHB was the first attempt to identify the genetic basis of firmness variation in a breeding population [141]. SNP-firmness associations were detected for tetraploid (five associations) and diploid (three associations) gene action models only under a less stringent threshold (q -value of 0.1). Out of those, two missense variants were identified, one at a gene encoding a ubiquitin-like-specific cysteine proteinase (CUFF.36470.1) and another at a gene encoding a S-adenosyl-L-methionine-dependent methyltransferase (SAM-MTase) (CUFF.1480.1). Cysteine proteinases have been shown to act as post-transcriptional regulators of ripening-related proteins in tomato [142] and cysteine proteinases were also differentially expressed between firm and soft strawberry cultivars [143]. SAM-MTases catalyze transmethylation reactions in various biomolecules, acting in the biosynthesis pathway of ethylene and polyamines, which also have important roles during the ripening process and may affect fruit firmness [144–148]. Hence, some of the significant SNPs were detected within biologically plausible candidate genes affecting the trait [141]. However, individual markers explained a small portion of the phenotypic variation (less than 3.46%). These results suggested that firmness is indeed a quantitative trait, whose phenotypic expression depends on the cumulative actions of many genes with small effects and their interaction with environment. However, assuming that firmness and crispness are correlated traits, these results might conflict with the results of Blaker (2013)[82], where crispness segregation pattern fit the expected ratio for a monogenic trait. Ferrão et al. (2018) [141] also raised the concern that controlling for population structure in the GWAS model can strongly reduce the statistical power to detect associations when phenotypes are correlated with relationship. In this scenario, a QTL mapping would be a more suitable approach for detecting loci with large effects.

Rather than seeking to identify loci significantly associated with a trait as in GWAS and QTL mapping approaches, GS uses all marker simultaneously to predict the breeding value of individuals [149]. Hence, selections based on GS predictions have the potential to accelerate the breeding program, which is especially useful in perennial crops, like blueberry. To our knowledge, no study has been published applying GS in blueberry to date.

4. Conclusions and perspectives

High fruit firmness values are important for the whole blueberry industry chain. Among the main benefits, firmness is a key trait for the wide implementation of machine harvesting for fresh market, which will reduce financial and labor concerns. Considerable genetic gains have been achieved throughout the history of blueberry breeding, with modern cultivars being, on average, firmer than first-released genotypes. Despite the importance of this trait, the number of genetic and molecular studies reported in the literature is still modest. Recent insights through RNA-seq and GWAS analyses highlighted the role of cell wall degrading enzymes, cysteine proteinase, and SAM-MTase in fruit firmness variation across developmental stages and across genotypes. For the implementation of marker-assisted selection in order to accelerate the breeding process, new experimental designs are required. More accurate measurements of the trait using higher number of berry samples per genotype, broader population panels for GWAS and/or a QTL mapping

approach have the potential to detect loci with large effects on the phenotypic variation. Another breakthrough in the plant breeding field is the CRISPR/Cas9 (and related variants) technologies. However, editing the blueberry genome for improved firmness faces two major challenges: I) detect a candidate region with large effects on fruit firmness to be edited and II) develop CRISPR/Cas9 and tissue culture protocols for the species. More studies in these areas have the potential to unlock an era of faster and more efficient breeding tools for this healthy and high-value fruit crop.

Supplementary Material: The following are available online, Table S1: Fruit firmness survey for four cultivated blueberry types.

Author Contributions: FC performed the literature survey of firmness values. LFVF and JB performed the data analyses and interpretation. FC, JB, LFVF, and PM wrote the paper. PM conceived and supervised writing. All authors approved the final manuscript.

Funding: This work was funded by the UF royalty fund generated by the licensing of blueberry cultivars.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Szajdek, A.; Borowska, E. J. Bioactive Compounds and Health-Promoting Properties of Berry Fruits: A Review. *Plant Foods Hum. Nutr.* **2008**, *63*, 147–156, doi:10.1007/s11130-008-0097-5.
2. Rodriguez-Mateos, A.; Feliciano, R. P.; Cifuentes-Gomez, T.; Spencer, J. P. E. Bioavailability of wild blueberry (poly) phenols at different levels of intake. *J. Berry Res.* **2016**, *6*, 137–148, doi:10.3233/JBR-160123.
3. Norberto, S.; Silva, S.; Meireles, M.; Faria, A.; Pintado, M.; Calhau, C. Blueberry anthocyanins in health promotion: A metabolic overview. *J. Funct. Foods* **2013**, *5*, 1518–1528, doi:10.1016/j.jff.2013.08.015.
4. The Food and Agriculture Organization of the United Nations -FAOSTAT. Available online: <http://www.fao.org/faostat/en/#data/QC> (accessed on May 17, 2018).
5. Gallardo, R. K.; Stafne, E. T.; DeVetter, L. W.; Zhang, Q.; Li, C.; Takeda, F.; Williamson, J.; Yang, W. Q.; Cline, W. O.; Beaudry, R. Blueberry Producers' Attitudes toward Harvest Mechanization for Fresh Market. *Horttechnology* **2018**, *28*, 10–16, doi: 10.21273/HORTTECH03872-17.
6. Ehlenfeldt, M. K.; Martin, R. B. A survey of fruit firmness in highbush blueberry and species-introgressed blueberry cultivars. *HortScience* **2002**, *37*, 386–389.
7. Olmstead, J. W.; Finn, C. E. Breeding highbush blueberry cultivars adapted to machine harvest for the fresh market. *Horttechnology* **2014**, *24*, 290–294.
8. Berger, C. N.; Sodha, S. V.; Shaw, R. K.; Griffin, P. M.; Pink, D.; Hand, P.; Frankel, G. Fresh fruit and vegetables as vehicles for the transmission of human pathogens.

- 472 *Environ. Microbiol.* **2010**, *12*, 2385–2397, doi:10.1111/j.1462-2920.2010.02297.x.
- 473 9. Ehlenfeldt, M. K. Fruit Firmness and Holding Ability in Highbush Blueberry—
474 Implications for Mechanical Harvesting. *Int. J. fruit Sci.* **2005**, *5*, 83–91,
475 doi:10.1300/J492v05n03_08.
- 476 10. Moggia, C.; Graell, J.; Lara, I.; González, G.; Lobos, G. A. Firmness at Harvest
477 Impacts Postharvest Fruit Softening and Internal Browning Development in
478 Mechanically Damaged and Non-damaged Highbush Blueberries (*Vaccinium*
479 *corymbosum* L.). *Front. Plant Sci.* **2017**, *8*, doi:10.3389/fpls.2017.00535.
- 480 11. Mehra, L. K.; MacLean, D. D.; Savelle, A. T.; Scherm, H. Postharvest Disease
481 Development on Southern Highbush Blueberry Fruit in Relation to Berry Flesh Type
482 and Harvest Method. *Plant Dis.* **2013**, *97*, 213–221, doi:10.1094/PDIS-03-12-0307-
483 RE.
- 484 12. Giongo, L.; Poncetta, P.; Loretto, P.; Costa, F. Texture profiling of blueberries
485 (*Vaccinium* spp.) during fruit development, ripening and storage. *Postharvest Biol.*
486 *Technol.* **2013**, *76*, 34–39, doi:10.1016/j.postharvbio.2012.09.004.
- 487 13. Gilbert, J. L.; Guthart, M. J.; Gezan, S. A.; Pisaroglo de Carvalho, M.;
488 Schwieterman, M. L.; Colquhoun, T. A.; Bartoshuk, L. M.; Sims, C. A.; Clark, D.
489 G.; Olmstead, J. W. Identifying Breeding Priorities for Blueberry Flavor Using
490 Biochemical, Sensory, and Genotype by Environment Analyses. *PLoS One* **2015**, *10*,
491 e0138494, doi:10.1371/journal.pone.0138494.
- 492 14. Saftner, R.; Polashock, J.; Ehlenfeldt, M.; Vinyard, B. Instrumental and sensory
493 quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biol.*
494 *Technol.* **2008**, *49*, 19–26, doi:10.1016/j.postharvbio.2008.01.008.
- 495 15. Blaker, K. M.; Olmstead, J. W. Stone cell frequency and cell area variation of crisp
496 and standard texture southern highbush blueberry fruit. *J. Am. Soc. Hortic. Sci.* **2014**,
497 *139*, 553–557.
- 498 16. Forney, C. F.; Kalt, W.; Jordan, M. A.; Vinqvist-Tymchuk, M. R.; Fillmore, S. A. E.
499 Blueberry and cranberry fruit composition during development. *J. Berry Res.* **2012**,
500 *2*, 169–177, doi:10.3233/JBR-2012-034.
- 501 17. Chen, H.; Cao, S.; Fang, X.; Mu, H.; Yang, H.; Wang, X.; Xu, Q.; Gao, H. Changes
502 in fruit firmness, cell wall composition and cell wall degrading enzymes in
503 postharvest blueberries during storage. *Sci. Hortic. (Amsterdam)*. **2015**, *188*, 44–48,
504 doi:10.1016/j.scienta.2015.03.018.
- 505 18. Wang, D.; Yeats, T. H.; Uluisik, S.; Rose, J. K. C.; Seymour, G. B. Fruit Softening:
506 Revisiting the Role of Pectin. *Trends Plant Sci.* **2018**, *23*, 302–310,
507 doi:10.1016/j.tplants.2018.01.006.

- 508 19. Cellon, C.; Amadeu, R. R.; Olmstead, J. W.; Mattia, M. R.; Ferrao, L. F. V.; Munoz,
509 P. R. Estimation of genetic parameters and prediction of breeding values in an
510 autotetraploid blueberry breeding population with extensive pedigree data. *Euphytica*
511 **2018**, *214*, 87, doi:10.1007/s10681-018-2165-8.
- 512 20. Bell, H. P.; Burchill, J. Flower development in the lowbush blueberry. *Can. J. Bot.*
513 **1955**, *33*, 251–258, doi: 10.1139/b55-018.
- 514 21. Gough, R. E. The highbush blueberry and its management. Food Products Press,
515 Binghamton, NY, 1994.
- 516 22. Chu, W.; Gao, H.; Chen, H.; Wu, W.; Fang, X. Changes in Cuticular Wax
517 Composition of Two Blueberry Cultivars during Fruit Ripening and Postharvest
518 Cold Storage. *J. Agric. Food Chem.* **2018**, *66*, 2870–2876,
519 doi:10.1021/acs.jafc.7b05020.
- 520 23. Godoy, C.; Monterubbianesi, G.; Tognetti, J. Analysis of highbush blueberry
521 (*Vaccinium corymbosum* L.) fruit growth with exponential mixed models. *Sci.*
522 *Hortic. (Amsterdam)*. **2008**, *115*, 368–376, doi:10.1016/j.scienta.2007.10.018.
- 523 24. Jorquera-Fontena, E.; Génard, M.; Franck, N. Analysis of blueberry (*Vaccinium*
524 *corymbosum* L.) fruit water dynamics during growth using an ecophysiological
525 model. *J. Hortic. Sci. Biotechnol.* **2017**, *92*, 646–659,
526 doi:10.1080/14620316.2017.1304810.
- 527 25. Janick, J.; Paull, R. E. *The encyclopedia of fruit and nuts*; CAB International, **2008**,
528 Wallingford, UK.
- 529 26. Liu, M.; Pirrello, J.; CHERVIN, C.; Roustan, J.-P.; Bouzayen, M. Ethylene control
530 of fruit ripening: revisiting the complex network of transcriptional regulation. *Plant*
531 *Physiol.* **2015**, pp.01361.2015, doi:10.1104/pp.15.01361.
- 532 27. Kumar, R.; Khurana, A.; Sharma, A. K. Role of plant hormones and their interplay
533 in development and ripening of fleshy fruits. *J. Exp. Bot.* **2013**, *65*, 4561–4575,
534 doi:10.1093/jxb/eru277.
- 535 28. Haji, T.; Yaegaki, H.; Yamaguchi, M. Softening of stony hard peach by ethylene and
536 the induction of endogenous ethylene by 1-aminocyclopropane-1-carboxylic acid
537 (ACC). *J. Japanese Soc. Hortic. Sci.* **2003**, *72*, 212–217, doi:10.2503/jjshs.72.212.
- 538 29. Hoeberichts, F. A.; Van Der Plas, L. H. ; Woltering, E. J. Ethylene perception is
539 required for the expression of tomato ripening-related genes and associated
540 physiological changes even at advanced stages of ripening. *Postharvest Biol.*
541 *Technol.* **2002**, *26*, 125–133, doi:10.1016/S0925-5214(02)00012-1.
- 542 30. Suzuki, A.; Kikuchi, T.; Aoba, K. Changes of ethylene evolution, ACC content,

- 543 ethylene forming enzyme activity and respiration in fruits of highbush blueberry. *J.*
 544 *Japanese Soc. Hortic. Sci.* **1997**, *66*, 23–27, doi: 10.2503/jjshs.66.23.
- 545 31. Ban, T.; Kugishima, M.; Ogata, T.; Shiozaki, S.; Horiuchi, S.; Ueda, H. Effect of
 546 ethephon (2-chloroethylphosphonic acid) on the fruit ripening characters of rabbiteye
 547 blueberry. *Sci. Hortic. (Amsterdam)*. **2007**, *112*, 278–281,
 548 doi:10.1016/j.scienta.2006.12.027.
- 549 32. Costa, D.; Pintado, M.; Almeida, D. P. F. Postharvest ethylene application affects
 550 anthocyanin content and antioxidant activity of blueberry cultivars. *ISHS Acta*
 551 *Horticulturae, X International Symposium on Vaccinium and Other Superfruits 1017*,
 552 **2012**, 525–530, doi:10.17660/ActaHortic.2014.1017.65.
- 553 33. Chiabrando, V.; Giacalone, G. Shelf-life extension of highbush blueberry using 1-
 554 methylcyclopropene stored under air and controlled atmosphere. *Food Chem.* **2011**,
 555 *126*, 1812–1816, doi:10.1016/j.foodchem.2010.12.032.
- 556 34. Wang, S.; Zhou, Q.; Zhou, X.; Wei, B.; Ji, S. The effect of ethylene absorbent
 557 treatment on the softening of blueberry fruit. *Food Chem.* **2018**, *246*, 286–294,
 558 doi:10.1016/j.foodchem.2017.11.004.
- 559 35. DeLong, J. M.; Prange, R. K.; Bishop, C.; Harrison, P. A.; Ryan, D. A. J. The
 560 influence of 1-MCP on shelf-life quality of highbush blueberry. *HortScience* **2003**,
 561 *38*, 417–418.
- 562 36. Deng, J.; Shi, Z.; Li, X.; Liu, H. Effects of cold storage and 1-methylcyclopropene
 563 treatments on ripening and cell wall degrading in rabbiteye blueberry (*Vaccinium*
 564 *ashei*) fruit. *Food Sci. Technol. Int.* **2014**, *20*, 287–298,
 565 doi:10.1177/1082013213483611.
- 566 37. Blaker, K. M.; Olmstead, J. W. Effects of Preharvest Applications of 1-
 567 Methylcyclopropene on Fruit Firmness in Southern Highbush Blueberry. *ISHS Acta*
 568 *Horticulturae, X International Symposium on Vaccinium and Other Superfruits 1017*,
 569 **2012**, 71–75, doi:10.17660/ActaHortic.2014.1017.5.
- 570 38. MacLean, D. D.; NeSmith, D. S. Rabbiteye blueberry postharvest fruit quality and
 571 stimulation of ethylene production by 1-methylcyclopropene. *HortScience* **2011**, *46*,
 572 1278–1281.
- 573 39. Sun, Y.; Hou, Z.; Su, S.; Yuan, J. Effects of ABA, GA3 and NAA on fruit
 574 development and anthocyanin accumulation in blueberry. *J. South China Agric.*
 575 *Univ.* **2013**, *34*, 6–11.
- 576 40. Buran, T. J.; Sandhu, A. K.; Azeredo, A. M.; Bent, A. H.; Williamson, J. G.; Gu, L.
 577 Effects of exogenous abscisic acid on fruit quality, antioxidant capacities, and
 578 phytochemical contents of southern high bush blueberries. *Food Chem.* **2012**, *132*,

- 579 1375–1381, doi:10.1016/j.foodchem.2011.11.124.
- 580 41. Brummell, D. A. Cell wall disassembly in ripening fruit. *Funct. Plant Biol.* **2006**, *33*,
581 103, doi:10.1071/FP05234.
- 582 42. Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. Molecular
583 biology of the cell. Chapter 19: Cell Junctions, Cell Adhesion, and the Extracellular
584 Matrix. **2002**, 4th ed., Garland Science, New York - NY.
- 585 43. Daher, F. B.; Braybrook, S. A. How to let go: pectin and plant cell adhesion. *Front.*
586 *Plant Sci.* **2015**, *6*, doi:10.3389/fpls.2015.00523.
- 587 44. Carpita, N. C.; Gibeaut, D. M. Structural models of primary cell walls in flowering
588 plants: consistency of molecular structure with the physical properties of the walls
589 during growth. *Plant J.* **1993**, *3*, 1–30, doi:10.1111/j.1365-313X.1993.tb00007.x.
- 590 45. Huber, D. J. Polyuronide degradation and hemicellulose modifications in ripening
591 tomato fruit. *Journal-American Soc. Hortic. Sci.* **1983**.
- 592 46. Cutillas-Iturralde, A.; Zarra, I.; Fry, S. C.; Lorences, E. P. Implication of persimmon
593 fruit hemicellulose metabolism in the softening process. Importance of xyloglucan
594 endotransglycosylase. *Physiol. Plant.* **1994**, *91*, 169–176, doi:10.1111/j.1399-
595 3054.1994.tb00415.x.
- 596 47. Brummell, D. A.; Schröder, R. Xylan metabolism in primary cell walls. *New Zeal. J.*
597 *For. Sci.* **2009**, *39*, 125–143.
- 598 48. Atkinson, R. G.; Sutherland, P. W.; Johnston, S. L.; Gunaseelan, K.; Hallett, I. C.;
599 Mitra, D.; Brummell, D. A.; Schröder, R.; Johnston, J. W.; Schaffer, R. J. Down-
600 regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water
601 loss in apple (*Malus x domestica*) fruit. *BMC Plant Biol.* **2012**, *12*, 129,
602 doi:10.1186/1471-2229-12-129.
- 603 49. Vicente, A. R.; Ortugno, C.; Rosli, H.; Powell, A. L. T.; Greve, L. C.; Labavitch, J.
604 M. Temporal Sequence of Cell Wall Disassembly Events in Developing Fruits. 2.
605 Analysis of Blueberry (*Vaccinium* Species). *J. Agric. Food Chem.* **2007**, *55*, 4125–
606 4130, doi:10.1021/jf063548j.
- 607 50. Hanson, E. J.; Beggs, J. L.; Beaudry, R. M. Applying calcium chloride postharvest to
608 improve highbush blueberry firmness. *HortScience* **1993**, *28*, 1033–1034.
- 609 51. Angeletti, P.; Castagnasso, H.; Miceli, E.; Terminiello, L.; Concellón, A.; Chaves,
610 A.; Vicente, A. R. Effect of preharvest calcium applications on postharvest quality,
611 softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*)
612 varieties. *Postharvest Biol. Technol.* **2010**, *58*, 98–103,
613 doi:10.1016/j.postharvbio.2010.05.015.

- 614 52. Buchanan, B. B.; Gruissem, W.; Jones, R. L. *Biochemistry & molecular biology of*
615 *plants*; American Society of Plant Physiologists, v. 40, Rockville-MD, 2000.
- 616 53. Lara, I.; García, P.; Vendrell, M. Modifications in cell wall composition after cold
617 storage of calcium-treated strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest*
618 *Biol. Technol.* **2004**, *34*, 331–339, doi:10.1016/j.postharvbio.2004.05.018.
- 619 54. Conway, W. S.; Sams, C. E. The effects of postharvest infiltration of calcium,
620 magnesium, or strontium on decay, firmness, respiration, and ethylene production in
621 apples. *J. Am. Soc. Hortic. Sci.* **1987**.
- 622 55. Conway, W. S. Possible Mechanisms by Which Postharvest Calcium Treatment
623 Reduces Decay in Apples. *Phytopathology* **1984**, *74*, 208, doi:10.1094/Phyto-74-
624 208.
- 625 56. Sams, C. E.; Conway, W. S.; Abbott, J. A.; Lewis, R. J.; Ben-Shalom, N. Firmness
626 and decay of apples following postharvest pressure infiltration of calcium and heat
627 treatment. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 623–627.
- 628 57. Cabanne, C.; Donèche, B. Changes in polygalacturonase activity and calcium
629 content during ripening of grape berries. *Am. J. Enol. Vitic.* **2001**, *52*, 331–335.
- 630 58. Bonomelli, C.; Ruiz, R. Effects of foliar and soil calcium application on yield and
631 quality of table grape cv. ‘Thompson Seedless’. *J. Plant Nutr.* **2010**, *33*, 299–314,
632 doi:10.1080/01904160903470364.
- 633 59. Ciccicarese, A.; Stellacci, A. M.; Gentile, G.; Rubino, P. Effectiveness of pre- and
634 post-veraison calcium applications to control decay and maintain table grape fruit
635 quality during storage. *Postharvest Biol. Technol.* **2013**, *75*, 135–141,
636 doi:10.1016/j.postharvbio.2012.08.010.
- 637 60. García, J. M.; Herrera, S.; Morilla, A. Effects of Postharvest Dips in Calcium
638 Chloride on Strawberry. *J. Agric. Food Chem.* **1996**, *44*, 30–33,
639 doi:10.1021/jf950334l.
- 640 61. Hernández-Muñoz, P.; Almenar, E.; Ocio, M. J.; Gavara, R. Effect of calcium dips
641 and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*).
642 *Postharvest Biol. Technol.* **2006**, *39*, 247–253,
643 doi:10.1016/j.postharvbio.2005.11.006.
- 644 62. Hernández-Muñoz, P.; Almenar, E.; Valle, V. Del; Velez, D.; Gavara, R. Effect of
645 chitosan coating combined with postharvest calcium treatment on strawberry
646 (*Fragaria×ananassa*) quality during refrigerated storage. *Food Chem.* **2008**, *110*,
647 428–435, doi:10.1016/j.foodchem.2008.02.020.
- 648 63. Gupta, V.; Estrada, A. D.; Blakley, I.; Reid, R.; Patel, K.; Meyer, M. D.; Andersen,

- 649 S. U.; Brown, A. F.; Lila, M. A.; Loraine, A. E. RNA-Seq analysis and annotation of
650 a draft blueberry genome assembly identifies candidate genes involved in fruit
651 ripening, biosynthesis of bioactive compounds, and stage-specific alternative
652 splicing. *Gigascience* **2015**, *4*, 5, doi:10.1186/s13742-015-0046-9.
- 653 64. Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henrissat, B. The
654 carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* **2013**, *42*,
655 doi:10.1093/nar/gkt1178.
- 656 65. Yin, Y.; Mao, X.; Yang, J.; Chen, X.; Mao, F.; Xu, Y. dbCAN: a web resource for
657 automated carbohydrate-active enzyme annotation. *Nucleic Acids Res.* **2012**, *40*,
658 W445–W451, doi:10.1093/nar/gks479.
- 659 66. Asif, M. H.; Lakhwani, D.; Pathak, S.; Gupta, P.; Bag, S. K.; Nath, P.; Trivedi, P. K.
660 Transcriptome analysis of ripe and unripe fruit tissue of banana identifies major
661 metabolic networks involved in fruit ripening process. *BMC Plant Biol.* **2014**, *14*,
662 316, doi:10.1186/s12870-014-0316-1.
- 663 67. Rose, J. K. .; Bennett, A. B. Cooperative disassembly of the cellulose–xyloglucan
664 network of plant cell walls: parallels between cell expansion and fruit ripening.
665 *Trends Plant Sci.* **1999**, *4*, 176–183, doi:10.1016/S1360-1385(99)01405-3.
- 666 68. Goulao, L. F.; Oliveira, C. M. Cell wall modifications during fruit ripening: when a
667 fruit is not the fruit. *Trends Food Sci. Technol.* **2008**, *19*, 4–25,
668 doi:10.1016/j.tifs.2007.07.002.
- 669 69. Chandrasekar, B.; van der Hoorn, R. A. L. Beta galactosidases in Arabidopsis and
670 tomato—a mini review. *Biochem. Soc. Trans.* **2016**, *44*, 150–158,
671 doi:10.1042/BST20150217.
- 672 70. Hossain, M. A.; Rana, M. M.; Kimura, Y.; Roslan, H. A. Changes in Biochemical
673 Characteristics and Activities of Ripening Associated Enzymes in Mango Fruit
674 during the Storage at Different Temperatures. *Biomed Res. Int.* **2014**, *2014*, 1–11,
675 doi:10.1155/2014/232969.
- 676 71. Uluisik, S.; Chapman, N. H.; Smith, R.; Poole, M.; Adams, G.; Gillis, R. B.; Besong,
677 T. M. D.; Sheldon, J.; Stiegelmeier, S.; Perez, L.; Samsulrizal, N.; Wang, D.; Fisk, I.
678 D.; Yang, N.; Baxter, C.; Rickett, D.; Fray, R.; Blanco-Ulate, B.; Powell, A. L. T.;
679 Harding, S. E.; Craigon, J.; Rose, J. K. C.; Fich, E. A.; Sun, L.; Domozych, D. S.;
680 Fraser, P. D.; Tucker, G. A.; Grierson, D.; Seymour, G. B. Genetic improvement of
681 tomato by targeted control of fruit softening. *Nat. Biotechnol.* **2016**, *34*, 950–952,
682 doi:10.1038/nbt.3602.
- 683 72. Wu, B.; Gao, L.; Gao, J.; Xu, Y.; Liu, H.; Cao, X.; Zhang, B.; Chen, K. Genome-
684 Wide Identification, Expression Patterns, and Functional Analysis of UDP
685 Glycosyltransferase Family in Peach (*Prunus persica* L. Batsch). *Front. Plant Sci.*

- 2017, 8, doi:10.3389/fpls.2017.00389.
73. CARBONE, F.; PREUSS, A.; DE VOS, R. C. H.; D'AMICO, E.; PERROTTA, G.; BOVY, A. G.; MARTENS, S.; ROSATI, C. Developmental, genetic and environmental factors affect the expression of flavonoid genes, enzymes and metabolites in strawberry fruits. *Plant. Cell Environ.* **2009**, 32, 1117–1131, doi:10.1111/j.1365-3040.2009.01994.x.
 74. Paniagua, A. C.; East, A. R.; Hindmarsh, J. P.; Heyes, J. A. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biol. Technol.* **2013**, 79, 13–19, doi:10.1016/j.postharvbio.2012.12.016.
 75. Moggia, C.; Beaudry, R. M.; Retamales, J. B.; Lobos, G. A. Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. *Postharvest Biol. Technol.* **2017**, 132, 88–96, doi:10.1016/j.postharvbio.2017.05.019.
 76. Moggia, C.; Graell, J.; Lara, I.; Schmeda-Hirschmann, G.; Thomas-Valdés, S.; Lobos, G. A. Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. *Sci. Hortic. (Amsterdam)*. **2016**, 211, 449–457, doi:10.1016/j.scienta.2016.09.018.
 77. Sun, X.; Narciso, J.; Wang, Z.; Ference, C.; Bai, J.; Zhou, K. Effects of Chitosan-Essential Oil Coatings on Safety and Quality of Fresh Blueberries. *J. Food Sci.* **2014**, 79, doi:10.1111/1750-3841.12447.
 78. Abugoch, L.; Tapia, C.; Plasencia, D.; Pastor, A.; Castro-Mandujano, O.; López, L.; Escalona, V. H. Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film. *J. Sci. Food Agric.* **2016**, 96, 619–626, doi:10.1002/jsfa.7132.
 79. Harker, F. R.; Redgwell, R. J.; Hallett, I. C.; Murray, S. H.; Carter, G. Chapter 2: Texture of fresh fruit. In *Horticultural Reviews*, Janick J. (ed), *Am. Soc. Hortic. Sci.* v.20, **1997**, pp.121–224, doi:10.1002/9780470650646.ch2.
 80. Allan-Wojtas, P. M.; Forney, C. F.; Carbyn, S. E.; Nicholas, K. U. K. G. Microstructural Indicators of Quality-related Characteristics of Blueberries—An Integrated Approach. *LWT - Food Sci. Technol.* **2001**, 34, 23–32, doi:10.1006/fstl.2000.0738.
 81. Fava, J.; Alzamora, S. M.; Castro, M. A. Structure and Nanostructure of the Outer Tangential Epidermal Cell Wall in *Vaccinium corymbosum* L. (Blueberry) Fruits by Blanching, Freezing-Thawing and Ultrasound. *Food Sci. Technol. Int.* **2006**, 12, 241–251, doi:10.1177/1082013206065702.
 82. Blaker, K. M. *Comparison of crisp and standard fruit texture in southern highbush*

- 722 *blueberry using instrumental and sensory panel techniques*; University of Florida,
723 2013.
- 724 83. Finn, C. E.; Luby, J. J. Inheritance of fruit quality traits in blueberry. *J. Am. Soc.*
725 *Hortic. Sci.* **1992**, *117*, 617–621.
- 726 84. Ali, S.; Zaman, Q. U.; Schumann, A. W.; Udenigwe, C. C.; Farooque, A. A. Impact
727 of fruit ripening parameters on harvesting efficiency of the wild blueberry harvester.
728 *ASABE Annual International Meeting*; American Society of Agricultural and
729 Biological Engineers, **2016**; doi:10.13031/aim.20162460762.
- 730 85. Vilela, A.; Gonçalves, B.; Ribeiro, C.; Fonseca, A. teresa; Correia, S.; Fernandes, H.;
731 Ferreira, S.; Bacelar, E.; Silva, A. P. Study of Textural, Chemical, Color and Sensory
732 Properties of Organic Blueberries Harvested in Two Distinct Years: A Chemometric
733 Approach. *J. Texture Stud.* **2016**, *47*, 199–207, doi:10.1111/jtxs.12173.
- 734 86. Concha-Meyer, A.; Eifert, J. D.; Williams, R. C.; Marcy, J. E.; Welbaum, G. E. Shelf
735 life determination of fresh blueberries (*Vaccinium corymbosum*) stored under
736 controlled atmosphere and ozone. *Int. J. food Sci.* **2015**, doi:10.1155/2015/164143.
- 737 87. Hu, M.-H.; Dong, Q.-L.; Liu, B.-L.; Opara, U. L. Prediction of mechanical properties
738 of blueberry using hyperspectral interactance imaging. *Postharvest Biol. Technol.*
739 **2016**, *115*, 122–131, doi:10.1016/j.postharvbio.2015.11.021.
- 740 88. Brazelton, D. M.; Wagner, A. L. Blueberry plant named ‘Last Call’ 2015. Available
741 at: <https://patents.google.com/patent/USPP25386P3>.
- 742 89. NeSmith, D. S. ‘Suziblu’ Southern Highbush Blueberry. *HortScience* **2010**, *45*,
743 142–143.
- 744 90. Grajkowski, J.; Ochman, I.; Muliński, Z. Firmness and antioxidant capacity of
745 highbush blueberry (*Vaccinium corymbosum* L.) grown on three types of organic
746 bed. *Veg. Crop. Res. Bull.* **2007**, *66*, 155–159, doi:10.2478/v10032-007-0017-5.
- 747 91. Blaker, K. M.; Plotto, A.; Baldwin, E. A.; Olmstead, J. W. Correlation between
748 sensory and instrumental measurements of standard and crisp-texture southern
749 highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids). *J. Sci. Food*
750 *Agric.* **2014**, *94*, 2785–2793, doi:10.1002/jsfa.6626.
- 751 92. NeSmith, D. S.; Draper, A. D.; Spiers, J. M. Palmetto’ Southern Highbush Blueberry.
752 *HortScience* **2004**, *39*, 1774–1775.
- 753 93. Paylis, G. C. Blueberry fruit quality and yield as affected by fertilization. *ISHS Acta*
754 *Horticulturae, VIII International Symposium on Vaccinium Culture 715*, **2004**, 353–
755 356, doi: 10.17660/ActaHortic.2006.715.52.
- 756 94. Ochmian, I.; Grajkowski, J.; Skupień, K. Influence of substrate on yield and

- 757 chemical composition of highbush blueberry fruit cv. 'Sierra'. *J. Fruit Ornam. Plant*
758 *Res.* **2009**, *17*, 89–100.
- 759 95. Retamales, J. B.; Lobos, G. A.; Romero, S.; Godoy, R.; Moggia, C. Repeated
760 applications of CPPU on highbush blueberry cv. Duke increase yield and enhance
761 fruit quality at harvest and during postharvest. *Chil. J. Agric. Res.* **2014**, *74*, 157–
762 161, doi:10.4067/S0718-58392014000200005.
- 763 96. Yu, P.; Li, C.; Takeda, F.; Krewer, G. Visual bruise assessment and analysis of
764 mechanical impact measurement in southern highbush blueberries. *Appl. Eng. Agric.*
765 **2014**, *30*, 29–37, doi:10.13031/aea.30.10224.
- 766 97. Stringer, S. J.; Draper, A. D.; Spiers, J. M.; Marshall, D. A.; Smith, B. J.
767 'Pearl' Southern Highbush Blueberry. *HortScience* **2013**, *48*, 130–131.
- 768 98. Lobos, G. A.; Callow, P.; Hancock, J. F. The effect of delaying harvest date on fruit
769 quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum*
770 L.). *Postharvest Biol. Technol.* **2014**, *87*, 133–139,
771 doi:10.1016/j.postharvbio.2013.08.001.
- 772 99. Ehret, D. L.; Frey, B.; Forge, T.; Helmer, T.; Bryla, D. R. Effects of drip irrigation
773 configuration and rate on yield and fruit quality of young highbush blueberry plants.
774 *HortScience* **2012**, *47*, 414–421.
- 775 100. Ochmian, I.; Grajkowski, J.; Skupieñ, K. Effect of substrate type on the field
776 performance and chemical composition of highbush blueberry cv. Patriot.
777 *Agricultural and Food Science*, **2010**, *19*, 69–80, doi:10.2137/145960610791015078.
- 778 101. Hicklenton, P.; Forney, C.; Domytrak, C. Use of row covers and post harvest storage
779 techniques to alter maturity and marketing period for highbush blueberries. ISHS
780 *Acta Horticulturae, XXVI International Horticultural Congress: Berry Crop*
781 *Breeding, Production and Utilization for a New Century* 626, **2002**, 287–295,
782 doi:10.17660/ActaHortic.2003.626.40.
- 783 102. Sargent, S. A.; Berry, A. D.; Brecht, J. K.; Santana, M.; Zhang, S.; Ristow, N.
784 Studies on quality of southern highbush blueberry cultivars: effects of pulp
785 temperature, impact and hydrocooling. ISHS *Acta Horticulturae, XI International*
786 *Vaccinium Symposium 1180*, **2016**, 497–502,
787 doi:10.17660/ActaHortic.2017.1180.70.
- 788 103. Brazelton, D. M.; Wagner, A. L. Blueberry plant named 'CLOCKWORK' 2013.
789 Available at: <https://patents.google.com/patent/US20130239264P1>.
- 790 104. Yang, W. Q.; Andrews, H. E.; Basey, A. Blueberry rootstock: selection, evaluation,
791 and field performance of grafted blueberry plants. ISHS *Acta Horticulturae, XXIX*
792 *International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods*

- 793 *and Landscapes* 1117, **2014**, 119–124, doi: 10.17660/ActaHortic.2016.1117.20.
- 794 105. Brazelton, D. M.; Wagner, A. L. Blueberry plant named ‘Cargo’ 2014. Available at:
795 <https://patents.google.com/patent/US20130239260P1>.
- 796 106. Brazelton, D. M.; Wagner, A. L. Blueberry plant named ‘Blue Ribbon’ 2014.
797 Available at: <https://patents.google.com/patent/US20130239265P1>.
- 798 107. Brazelton, D. M.; Wagner, A. L. Blueberry plant named ‘Top Shelf’ 2014. Available
799 at: <https://patents.google.com/patent/US20130239261P1>.
- 800 108. Lobos, G. A.; Bravo, C.; Valdés, M.; Lara, I.; Graell, J.; Moggia, C.; González, C.
801 Changes in quality and maturity of ‘Duke’ and ‘Brigitta’ blueberries during fruit
802 development: postharvest implications. *ISHS Acta Horticulturae, VIII International*
803 *Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits-Ethical*
804 *and Technological Issues* 1194, **2016**, 1495–1501,
805 doi:10.17660/ActaHortic.2018.1194.209.
- 806 109. Patel, N. Blueberry plant named ‘Hortblue Poppins’ 2011. Available at:
807 <https://patents.google.com/patent/USPP21881P3>.
- 808 110. Ballington, J. R.; Bland, W. T. Blueberry plant named ‘Heintooga’ 2017. Available
809 at: <https://patents.google.com/patent/US20170188494P1>.
- 810 111. Strik, B. C.; Vance, A. J.; Finn, C. E. Northern Highbush Blueberry Cultivars
811 Differed in Yield and Fruit Quality in Two Organic Production Systems from
812 Planting to Maturity. *HortScience* **2017**, 52, 844–851,
813 doi:10.21273/HORTSCI11972-17.
- 814 112. Rodríguez-Armenta, H. P.; Olmstead, J. W.; Lyrene, P. M. Characterization of
815 backcross blueberry populations created to introgress *Vaccinium arboreum* traits into
816 southern highbush blueberry. *ISHS Acta Horticulturae, XI International Vaccinium*
817 *Symposium* 1180, **2016**, 435–444, doi:10.17660/ActaHortic.2017.1180.61.
- 818 113. Ballington, J.; Rooks, S. Blueberry named ‘Carteret’ 2007. Available at:
819 <https://patents.google.com/patent/US20070143882>.
- 820 114. Ballington, J.; Rooks, S. Blueberry named ‘New Hanover’ 2007. Available at:
821 <https://patents.google.com/patent/USPP19990P3>.
- 822 115. Ballington, J. R.; Rooks, S. D. Blueberry named ‘Robeson’ 2009. Available at:
823 <https://patents.google.com/patent/USPP19756P3>.
- 824 116. F. Almutairi, K.; Bryla, D. R.; Strik, B. C. Potential of Deficit Irrigation, Irrigation
825 Cutoffs, and Crop Thinning to Maintain Yield and Fruit Quality with Less Water in
826 Northern Highbush Blueberry. *HortScience* **2017**, 52, 625–633,
827 doi:10.21273/HORTSCI11533-16.

- 828 117. Strik, B.; Buller, G. Nitrogen fertilization rate, sawdust mulch, and pre-plant
829 incorporation of sawdust-long-term impact on yield, fruit quality, and soil and plant
830 nutrition in 'elliott'. *ISHS Acta Horticulturae, X International Symposium on*
831 *Vaccinium and Other Superfruits 1017*, **2012**, 269–275, doi:
832 10.17660/ActaHortic.2014.1017.34.
- 833 118. NeSmith, D. S.; Prussia, S.; Tetteh, M.; Krewer, G. Firmness losses of rabbiteye
834 blueberries (*Vaccinium ashei* Reade) during harvesting and handling. *ISHS Acta*
835 *Horticulturae, VII International Symposium on Vaccinium Culture 574*, **2000**, 287–
836 293, doi:10.17660/ActaHortic.2002.574.43.
- 837 119. Yang, W. Q.; Harpole, J.; Finn, C. E.; Strik, B. C. Evaluating berry firmness and
838 total soluble solids of newly released highbush blueberry cultivars. *ISHS Acta*
839 *Horticulturae, IX International Vaccinium Symposium 810*, **2008**, 863–868,
840 doi:10.17660/ActaHortic.2009.810.115.
- 841 120. Li, C.; Luo, J.; MacLean, D. A novel instrument to delineate varietal and harvest
842 effects on blueberry fruit texture during storage. *J. Sci. Food Agric.* **2011**, *91*, 1653–
843 1658, doi:10.1002/jsfa.4362.
- 844 121. NeSmith, D. S.; Draper, A. D. 'Camellia' southern highbush blueberry. *J. Am.*
845 *Pomol. Soc.* **2007**, *61*, 34.
- 846 122. NeSmith, D. S.; Nunez-Barrios, A.; Prussia, S. E.; Aggarwal, D. Postharvest berry
847 quality of six rabbiteye blueberry cultivars in response to temperature. *J. Am. Pomol.*
848 *Soc.* **2005**, *59*, 13.
- 849 123. Nunez-Barrios, A.; Nesmith, D. S.; Chinnan, M.; Prussia, S. E. Dynamics of
850 rabbiteye blueberry fruit quality in response to harvest method and postharvest
851 handling temperature. *Small Fruits Rev.* **2005**, *4*, 73–81,
852 doi:10.1300/J301v04n02_08.
- 853 124. Ballington, J. R.; Ballinger, W. E.; Mainland, C. M.; Swallow, W. H.; Maness, E. P.
854 Ripening period of *Vaccinium* species in southeastern North Carolina [Blueberry,
855 breeding for both early-and late-ripening *Vaccinium* genotypes]. *J. Am. Soc. Hortic.*
856 *Sci.* **1984**.
- 857 125. Boches, P.; Bassil, N. V.; Rowland, L. Genetic diversity in the highbush blueberry
858 evaluated with microsatellite markers. *J. Am. Soc. Hortic. Sci.* **2006**, *131*, 674–686.
- 859 126. Hancock, J. F.; Lyrene, P.; Finn, C. E.; Vorsa, N.; Lobos, G. A. Blueberries and
860 cranberries. In *Temperate fruit crop breeding*, James F. Hancock (ed); Springer,
861 **2008**; pp. 115–150.
- 862 127. Goldy, R. G.; Lyrene, P. M. In vitro colchicine treatment of 4x blueberries,
863 *Vaccinium* sp.[Polyploid induction, genotype effect]. *J. Am. Soc. Hortic. Sci.* **1984**.

- 864 128. Chavez, D. J.; Lyrene, P. M. Effects of self-pollination and cross-pollination of
865 *Vaccinium darrowii* (Ericaceae) and other low-chill blueberries. *HortScience* **2009**,
866 *44*, 1538–1541.
- 867 129. Ehlenfeldt, M. K.; Draper, A. D.; Clark, J. R. Performance of southern highbush
868 blueberry cultivars released by the US Department of Agriculture and cooperating
869 state agricultural experiment stations. *Horttechnology* **1995**, *5*, 127–130.
- 870 130. Coville, F. V. Improving the wild blueberry. In *Yearbook of Agriculture*, U.S.
871 Department of Agriculture, U.S. Government Printing Office: Washington, DC,
872 **1937**; 559–574.
- 873 131. Sharpe, R. H.; Sherman, W. B. “Flordablue’and’Sharpblue”: two new blueberries for
874 central Florida. *Circ Fla Coop Ext Serv Inst Food Agric Sci Univ Fla* **1976**.
- 875 132. Fillion, L.; Kilcast, D. Consumer perception of crispness and crunchiness in fruits
876 and vegetables. *Food Qual. Prefer.* **2002**, *13*, 23–29, doi: 10.1016/S0950-
877 3293(01)00053-2.
- 878 133. Aalders, L. E.; Hall, I. V. A study of variation in fruit yield and related characters in
879 two diallels of the lowbush blueberry, *Vaccinium angustifolium* Ait. *Can. J. Genet.*
880 *Cytol.* **1975**, *17*, 401–404, doi: 10.1139/g75-053.
- 881 134. Erb, W. A.; Draper, A. D.; Galletta, G. J.; Swartz, H. J. Combining ability for plant
882 and fruit traits of interspecific blueberry progenies on mineral soil. *J. Am. Soc.*
883 *Hortic. Sci.* **1990**, *115*, 1025–1028.
- 884 135. Scalzo, J.; Sguigna, V.; Mezzetti, B.; Stanley, J.; Alspach, P. Variation of fruit traits
885 in highbush blueberry seedlings from a factorial cross. *ISHS Acta Horticulturae*,
886 *XXVIII International Horticultural Congress on Science and Horticulture for People*
887 *926*, **2010**, 79–83, doi:10.17660/ActaHortic.2012.926.9.
- 888 136. Scalzo, J.; Stanley, J.; Alspach, P.; Mezzetti, B. Preliminary evaluation of fruit traits
889 and phytochemicals in a highbush blueberry seedling population. *J. Berry Res.* **2013**,
890 *3*, 103–111, doi:10.3233/JBR-130049.
- 891 137. Connor, A. M.; Luby, J. J.; Tong, C. B. S.; Finn, C. E.; Hancock, J. F. Genotypic and
892 environmental variation in antioxidant activity, total phenolic content, and
893 anthocyanin content among blueberry cultivars. *J. Am. Soc. Hortic. Sci.* **2002**, *127*,
894 89–97.
- 895 138. Finn, C. E.; Hancock, J. F.; Mackey, T.; Serce, S. Genotype× environment
896 interactions in highbush blueberry (*Vaccinium* sp. L.) families grown in Michigan
897 and Oregon. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 196–200.
- 898 139. Isik, F.; Holland, J.; Maltecca, C. *Genetic data analysis for plant and animal*

- 899 *breeding*, v.1, Springer, New York-NY, **2017**.
- 900 140. Hamblin, M. T.; Buckler, E. S.; Jannink, J.-L. Population genetics of genomics-
901 based crop improvement methods. *Trends Genet.* **2011**, *27*, 98–106,
902 doi:10.1016/j.tig.2010.12.003.
- 903 141. Ferrão, L. F. V; Benevenuto, J.; Oliveira, I. de B.; Cellon, C.; Olmstead, J.; Kirst,
904 M.; Resende, M. F. R.; Munoz, P. Insights into the genetic basis of blueberry fruit-
905 related traits using diploid and polyploid models in a GWAS context. *Front. Ecol.*
906 *Evol.* **2018**, *6*, 107, doi:10.3389/fevo.2018.00107.
- 907 142. Wang, W.; Cai, J.; Wang, P.; Tian, S.; Qin, G. Post-transcriptional regulation of fruit
908 ripening and disease resistance in tomato by the vacuolar protease SIVPE3. *Genome*
909 *Biol.* **2017**, *18*, 47, doi:10.1186/s13059-017-1178-2.
- 910 143. Salentijn, E. M. J.; Aharoni, A.; Schaart, J. G.; Boone, M. J.; Krens, F. A.
911 Differential gene expression analysis of strawberry cultivars that differ in fruit-
912 firmness. *Physiol. Plant.* **2003**, *118*, 571–578, doi:10.1034/j.1399-
913 3054.2003.00138.x.
- 914 144. Roje, S. S-Adenosyl-l-methionine: Beyond the universal methyl group donor.
915 *Phytochemistry* **2006**, *67*, 1686–1698, doi:10.1016/j.phytochem.2006.04.019.
- 916 145. Paul, V.; Pandey, R.; Srivastava, G. C. The fading distinctions between classical
917 patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of
918 ethylene—An overview. *J. Food Sci. Technol.* **2012**, *49*, 1–21, doi:10.1007/s13197-
919 011-0293-4.
- 920 146. Van de Poel, B.; Bulens, I.; Oppermann, Y.; Hertog, M. L. A. T. M.; Nicolai, B. M.;
921 Sauter, M.; Geeraerd, A. H. S -adenosyl- l -methionine usage during climacteric
922 ripening of tomato in relation to ethylene and polyamine biosynthesis and
923 transmethylation capacity. *Physiol. Plant.* **2013**, *148*, 176–188, doi:10.1111/j.1399-
924 3054.2012.01703.x.
- 925 147. Singh, R.; Rastogi, S.; Dwivedi, U. N. Phenylpropanoid Metabolism in Ripening
926 Fruits. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 398–416, doi:10.1111/j.1541-
927 4337.2010.00116.x.
- 928 148. Moffatt, B. A.; Weretilnyk, E. A. Sustaining S-adenosyl-l-methionine-dependent
929 methyltransferase activity in plant cells. *Physiol. Plant.* **2001**, *113*, 435–442, doi:
930 10.1034/j.1399-3054.2001.1130401.x.
- 931 149. Jannink, J.-L.; Lorenz, A. J.; Iwata, H. Genomic selection in plant breeding: from
932 theory to practice. *Brief. Funct. Genomics* **2010**, *9*, 166–177,
933 doi:10.1093/bfpg/elq001.