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Research Report

Accumulation of sugars and associated gene expression in highbush blueberries differ by cultivar, ripening stage, and storage temperature

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Abstract.
BACKGROUND: The sweetness of highbush blueberries is a sensory quality standard for consumers. Changes in metabolites and expression of associated genes induce variation in the sensory quality of blueberries.
OBJECTIVE: This work investigated the ripening mechanism of blueberries by measuring changes in organic acids and sugar content as well as the gene expression levels associated with sugar accumulation, such as β amylase, invertase, sucrose phosphate synthase, and tonoplast monosaccharide transporter genes, before and after harvesting.
METHODS: We used three different blueberry cultivars (Duke, Sierra, and Elizabeth) that we harvested at two ripening stages in a randomized complete block design and then kept at room temperature or 4°C for 7 days. The organic acids and sugar content were measured via HPLC, and gene expression levels were analyzed by qPCR.
RESULTS: Cultivars, ripening stage, and storage conditions affected the synthesis of sugars and degradation of organic acids to determine the sweetness in blueberries and the expression of tagged genes and analyses of compounds involved in the metabolic mechanism.
CONCLUSIONS: The results provide insights into the mechanism underlying the ripening and the postharvest quality. This study may support the selection of suitable blueberry cultivars that meet customer demand.

Keywords: Blueberries, gene expression levels, ripening, storage conditions, sugars

1. Introduction

Highbush blueberries (*Vaccinium corymbosum* L.) are among the most popular fruits among consumers because of their wide range of health benefits [1] and sweet taste [2]. The flavor is essentially a balance between sugar and

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acid, and the acidity and sweetness of blueberries play an essential role in their flavor characteristics. Blueberry flavor varies according to cultivar [3, 4], ripening [5], and postharvest storage conditions [6, 7].

Organic acids and carbohydrates (sugars) play an essential role in general fruit metabolism, and they determine the quality, sweetness, and tartness of blueberries [8, 9]. There is a decrease in organic acid content and increased sugar content [10, 11]. Among the organic acids, citric acid accounts for the most acid content in blueberries [12]. Glucose and fructose are the two main reducing sugars in blueberries, determining the fruit's sweetness [5, 9]. The levels of these sugars, critical for postharvest products, are affected by handling and storage conditions [13, 14].

Metabolism of carbohydrates and organic acids is essential in both the preharvest and postharvest shelf life of fruit. Metabolic processes for the synthesis, degradation and transport of these compounds regulate the increase in sugar and decrease in organic acids [5, 15]. Starch, sucrose, and reducing sugars are the main compounds that involve sugar metabolism during the ripening of fruit. Sugar metabolism is associated with gene encoding expression for enzymes such as sucrose synthase, sucrose phosphate synthase, invertase [16–18], and β amylase [19, 20]. Common sugar transporter proteins, such as the tonoplast monosaccharide transporter, are also involved in the ripening and accumulation of sugars [21, 22]. Gene expression continues to change after harvesting, and fruit metabolites are affected by controlled storage conditions. Postharvest carbon dioxide treatment induces a decrease in the invertase gene expression in strawberry fruit [23]. Storage temperature causes changes in this gene's expression in postharvest pear [24] and fresh-cut melon [25]. Thus, a controlled storage environment is critical for maintaining the postharvest quality of blueberries [26–28]. However, gene expression dynamics in the synthesis, transport, and degradation of sugars in different ripening stages and postharvest blueberries are still unclear.

Among highbush blueberries, Duke is an early-ripening cultivar with good taste and large fruit [29]. Elizabeth has a sweeter taste than Duke and is a late-ripening one, and Sierra is a small and sour fruit and its harvest time between Duke and Elizabeth. People do not recommend Sierra for commercial production because of its poor quality [30]. The typical signal of ripening blueberries is the change in skin color, and their surface turns dark blue to indicate the appropriate HT. However, after this color change, the flesh may be unripe or overripe, depending on the cultivar. The ripening process of blueberries is also variable during the period from harvesting until distribution to customers. During the distribution period, the fruit may be stored at low temperatures or distributed at ambient temperature, and these conditions also affect the ripening and quality of blueberries [31, 32]. Therefore, choosing the appropriate HT for different blueberry cultivars is critical for improving the postharvest quality of fruit [33, 34]. However, understanding the metabolic mechanism between sugars and acids in these cultivars is limited. For example, which factors induce the sour taste of Sierra and the sweet taste of Elizabeth, and why changes in sugar and acid metabolites differ among cultivars during ripening, after harvest, and during postharvest handling remain unclear. Which genes are involved in these mechanisms also require further study?

Thus, we investigated the ripening and metabolic mechanisms of blueberries, considering the changes in the contents of organic acids, sugars, and expression levels of *VcBAM*, *VcINV*, *VcSPS*, and *VcTMT*. Three different blueberry cultivars were studied (Duke, Sierra, and Elizabeth) at two ripening stages and two storage temperatures, room temperature (RT) and 4°C for 7 days. The results will support studies on metabolic mechanisms in blueberries during ripening and postharvest storage and support cultivation practices.

2. Materials and methods

2.1. Fruit materials

Three cultivars of blueberry (Duke, Sierra, and Elizabeth) were harvested at two stages of ripening (stage 3: unripe fruit and stage 5: ripe fruit) (Supplementary Figures 2, 3) in a randomized complete block design. Damaged free fruit, with similar size and color in each block, were sampled right after HT for analyses (initial

samples at each ripening stage) and were stored for 7 days at 4°C (4C) or stored for 7 days at room temperature (RT). Stored fruit was kept in polyethylene tubes and at 4°C or RT. After 7 days, the fruit was immediately sampled for analyses of soluble solid content (SSC), and sugars and organic acids were extracted. Other samples were stored at -80°C for RNA isolation and analyses of gene expression levels.

2.2. Analyses of organic acids

Organic acid contents were detected by the Ultimate 3000 High-Performance Liquid Chromatography (HPLC) System (Thermo Dionex, Sunnyvale, CA, USA) with a refractive index (RI) ultraviolet detector (RefractoMAX520; ERC Inc., Kawaguchi, Japan) with a wavelength of 210 nm. The extraction method was modified according to previous studies [8, 35], and the HPLC method was described by Chea et al. (2019). About 5–10 g frozen samples were macerated in 25 mL bi-distilled water using the Polytron P-10 tissue homogenizer (Brinkmann, Sayville, NY, USA). The slurry samples were centrifuged at 14000 *g* at 4°C for 10 min. After filtration with filtered paper (200 mm; Whatman, Maidstone, UK) and 0.45 μm microporous membranes, the collected supernatants were kept at –20°C for HPLC analyses. Chromatographic separation was performed using the Aminex 87H column (9 μm, 300 × 10 mm; Bio-Rad, Hercules, CA, USA). The analyses were performed at a flow rate of 0.5 mL/min. The mobile phase consisted of 0.01 N sulfuric acid (Fluka Chemicals, St. Louis, MO, USA). Detected organic acids (citric acid, quinic acid, and shikimic acid) were calculated based on their standards and expressed in g/kg fresh weight.

2.3. Analyses of individual sugars

Individual sugar content was detected by HPLC (Ultimate 3000; Thermo Dionex) with a RI detector, namely Shodex RI-101 with Chromeleon 6.0 software (Shodex, Yokohama, Japan). The analyses were modified from previous studies [8, 35]. About 5–10 g frozen ground tissue was mixed with 25 mL bi-distilled water using the Polytron P-10 tissue homogenizer (Brinkmann). The slurry samples were centrifuged at 14000 *g* at 4°C for 10 min. After filtration with filtered paper (200 mm; Whatman) and 0.45 μm microporous membranes, the collected supernatants were kept at –20°C for HPLC analyses. The Sugar-Pak column (300*6.5 mm; Waters Co., Milford, MA, USA) was used, the mobile phase consisted of bi-distilled water, the flow rate was 0.5 mL min^{-1}, the oven temperature was 70°C, and the injection volume was 10 μL. The individual sugars detected were calculated based on their standards such as glucose (98%; Junsei Chemical Co., Ltd., Tokyo, Japan), fructose (99%; Sigma, St. Louis, MO, USA), and sucrose (99.5%; Sigma). The values of sugar contents are expressed as g/kg fresh weight (FW).

2.4. Evaluation of soluble solids content of fruit

SSC was assessed using a digital refractometer (GMK-701R; G-Won Hitech, Seoul, Korea) and expressed as a percentage [36]. Juice from fruit samples (50 g) from each biological repeat from each block group was extracted and measured.

2.5. Isolation of total RNA and preparation of cDNA

Total RNA was extracted from 100 mg frozen fruit powder using the Ribospin TM Seed/Fruit RNA mini commercial kit (GeneAll Biotechnology, Seoul, Korea) [37]. RNA was collected in 30 μL nuclease-free water after treatment with DNase I (GeneAll Biotechnology), and stored at –80°C. The concentrations of RNA samples were assessed using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA), and their integrity was evaluated by electrophoresis using a 2% gel. The first strand of cDNA was synthesized using

the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The cDNA was diluted 10-fold with DEPC-treated water and stored at –20°C until analyses.

2.6. Analyses of gene expression

The transcript levels of *VcBAM* (Genbank Accession No. JQ911593), *VcINV*, *VcSPS*, and *VcTMT* were analyzed using real-time quantitative polymerase chain reaction (qPCR). Specific gene primers were designed using the National Center for Biotechnology Information (NCBI) primer blast tool (https://www.ncbi.nlm.nih.gov/). The PCR products were confirmed by electrophoresis, gel purification, and sequencing (Supplementary Figure 1). Partial coding sequences of *VcINV*, *VcSPS*, and *VcTMT* from blueberry databases (https://www.vaccinium.org/crop/blue berry) were confirmed by sequencing (Supplementary Figure 4) and deposited into NCBI (Accession Nos. MW383486, MT920322, and MT912540 respectively (Supplementary Table 1). The qPCR reactions were performed with the Mx3005P qPCR System (Agilent Technologies, Santa Clara, CA, USA) using Solgent™ 2X Real-Time Smart mix. A total reaction volume of 10 μL was used, including 2 μL cDNA template which was performed in accordance with the manufacturer's instructions (SolGent, Daejeon, Korea) [38]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as the internal control [39]. The thermal cycling parameters were 95°C for 10 min with 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The $2^{-\Delta\Delta CT}$ method was used to normalize and calibrate transcript values relative to GAPDH as previously described [40]. For each experiment, three independent replicates of the qPCR assays were performed.

2.7. Statistical analyses

The data mean and the Pearson correlation data were statistically evaluated using SAS 9.4 (TS1M2) statistical software (SAS Institute Inc., Cary, NC, USA). The experiment was performed in triplicate, and data are expressed as means ± standard deviations. Figures were generated using Sigma plot 12.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Effects of ripening and storage conditions on organic acids

Organic acids tend to decrease as blueberries are ripening (Fig. 1). Citric acid content decreased from more than 20 g/kg fresh weight (FW) to ∼10 g/kg FW in Duke and from more than 15 g/kg FW to ∼7 g/kg FW in Sierra and Elizabeth (Fig. 1A, B). Likewise, quinic acid content was significantly lower in ripe fruit (∼4 g/kg FW vs. ∼2 g/kg FW) in all cultivars (Fig. 1C, D). However, shikimic acid levels did not decrease significantly as Duke and Elizabeth ripe (Fig. 1E, F). The effects of storage conditions on organic acid content in postharvest fruit depended on the cultivar, ripening stage, and type of acid. Cold storage (4°C) did not induce significant changes in citric acid contents in both unripe and ripe blueberries. RT conditions caused a decline in citric acid content in Duke and Elizabeth, but RT did not induce changes in this acid in Sierra at both ripening stages. Citric acid contents decreased from 21.02 to 17.99 g/kg FW in Duke and 17.57 to 10.39 g/kg FW in Elizabeth in the unripe stage. This acid content decreased from 10.44 to 7.03 g/kg FW in Duke and from 8.10 to 5.06 g/kg FW in Elizabeth in the ripe stage (Fig. 1A, B). Storage conditions did not induce significant changes in quinic acid content in Sierra, but they rendered a considerable decrease in quinic acid contents in Elizabeth at 4°C and in Duke at RT in both unripe and ripe fruit (Fig. 1 C, D). Interestingly, cold storage induced a decrease in quinic acid content in un-ripe Duke (from 4.68 to 3.75 g/kg FW) and RT induced this decrease in un-ripe Elizabeth (from 2.01 to 1.58 g/kg FW) (Fig. 1C). The reduction of shikimic acid was not influenced by cold storage in Duke and Elizabeth for both unripe and ripe fruit, but cold storage caused the significant decline of shikimic acid

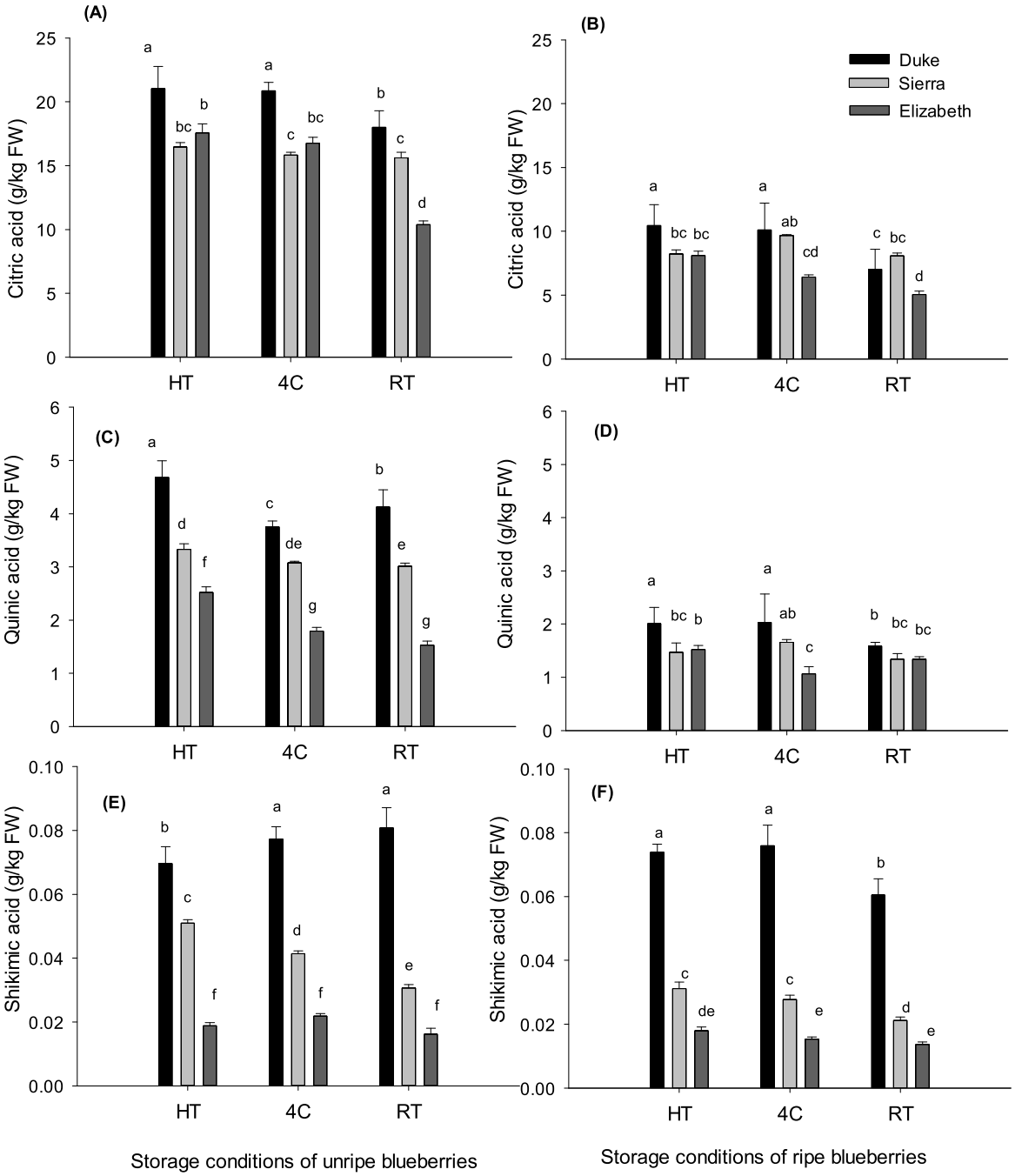

Fig. 1. Changes in the content of organic acids (A, B) citric acid, (C, D) quinic acid, and (E, F) shikimic acid in three blueberry fruit cultivars (Duke, Sierra, and Elizabeth) in un-ripe and ripe blueberries. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

in unripe Sierra (from 0.05 to 0.04 g/kg FW) (Fig. 1E, F). RT induced the decrease of shikimic acid content in Sierra in both unripe and ripe fruit, but this decrease only happened in ripe Duke. RT did not affect the changes in shikimic in Elizabeth. Interestingly, shikimic acid increased in both storage conditions in unripe Duke (from 0.06 to 0.07 g/kg FW at 4°C and from 0.06 to 0.08 g/kg FW at RT). Thus, organic acid content decreased as blueberries ripen, and the decrease depended on tagged acids, cultivars, and storage conditions.

3.2. Effects of ripening and storage conditions on individual sugars

Individual sugars (sucrose, glucose, and fructose) tend to increase as blueberries are ripe, depending on the type of sugars, cultivars, and storage conditions. In this study, we did not detect sucrose in ripe fruit of Duke and Elizabeth stored at RT, but sucrose appeared in Sierra and other samples of Elizabeth (Fig. 2A, B). Sucrose content was lower in ripe Elizabeth fruit at HT (1.04 g/kg FW unripe vs. 0.19 g/kg FW ripe). Glucose and fructose levels significantly increased during ripening in all three cultivars at HT (Fig. 2 C, D, E, F). However, storage conditions induced significant changes in these individual sugars and also depended on cultivars and ripening. Sucrose content decreased significantly in Elizabeth. In unripe Elizabeth fruit, sucrose levels were 0.53 g/kg FW at RT and 0.69 g/kg FW at 4°C. However, in ripe Elizabeth fruit, levels were higher at 4°C (0.47 g/kg FW) compared to HT (0.19 g/kg FW) and disappeared at RT (Fig. 2 A, B). Cold storage maintained glucose content in almost all samples, except to ripe Sierra (from 41.93 to 38.02 g/kg FW). RT induced a significant increase in glucose content Elizabeth and a significant decrease in Sierra at both unripe and ripe (Fig. 2 C, D). This glucose content increased from 24.19 to 28.59 g/kg FW in unripe Elizabeth and from 43.89 to 51.61 g/kg FW in ripe Elizabeth. However, this content decreased from 24.81 to 18.03 g/kg in unripe Sierra; from 41.93 to 36.49 g/kg FW in ripe Sierra. RT induced a significant increase in fructose contents in all cultivars for both ripening stages, but cold storage only induced the increase in fructose in Elizabeth at both ripening (from 28.47 to 34.85 g/kg FW in unripe; from 50.23 to 54.66 g/kg FW) and in ripe Duke (from 50.33 to 54.89 g/kg FW). The cold condition did not affect fructose content in Sierra. Summary: The Sierra cultivar had lower levels than the other cultivars in both ripening stages and storage conditions.

3.3. Effects of ripening and storage conditions on soluble solids

The increase of SSC in blueberries changed according to maturity and storage conditions but depended on the cultivar (Fig. 3A, B). SSC increased as fruit ripening (from 8.58% to 10% in Duke; 8.45% to 10.05% in Sierra; from 9.78% to 12.35% in Elizabeth). Elizabeth always had a higher SSC than the other cultivars. Storage conditions induced the increase of SSC, depending on cultivars and ripening. In unripe fruit, the SSC of Duke and Sierra were not changed significantly at 4°C, but this content increased significantly in Elizabeth in the same storage condition (from 9.78% to 10.41%). RT induced a significant increase of SSC in all tagged cultivars. In the ripe fruit, the SSC percentage of Duke was significantly higher than HT at both storage conditions. Both stored conditions did not affect changes in SSC of ripe Elizabeth, but cold storage improved the percentage of SSC in ripe Sierra.

3.4. Effects of ripening and storage conditions on the expression levels of tagged transcripts

Gene expression showed changes in the cultivars and storage conditions. The expression levels of the *VcBAM* gene unchanged as all tagged blueberries ripe (Fig. 4). The expression levels of *VcBAM* significantly increased in fruit stored at 4°C in almost all samples, except for ripe Sierra fruit. *VcBAM* was upregulated 11.23-fold in ripe fruit and 7.39-fold in unripe fruit in Duke at 4°C (Fig. 4A). This increase reached 39-fold in ripe and 16-fold in unripe Elizabeth (Fig. 4C). This transcript also increased 8.7-fold in unripe Sierra stored at 4°C (Fig. 4B).

The expression levels of *VcINV* only increased in Elizabeth when fruit was ripe (Fig. 5). It rose from 1 fold to 1.9 fold (Fig. 5C). There was a discrepancy in the genetic modification of *VcINV* between Sierra (Fig. 5B) and

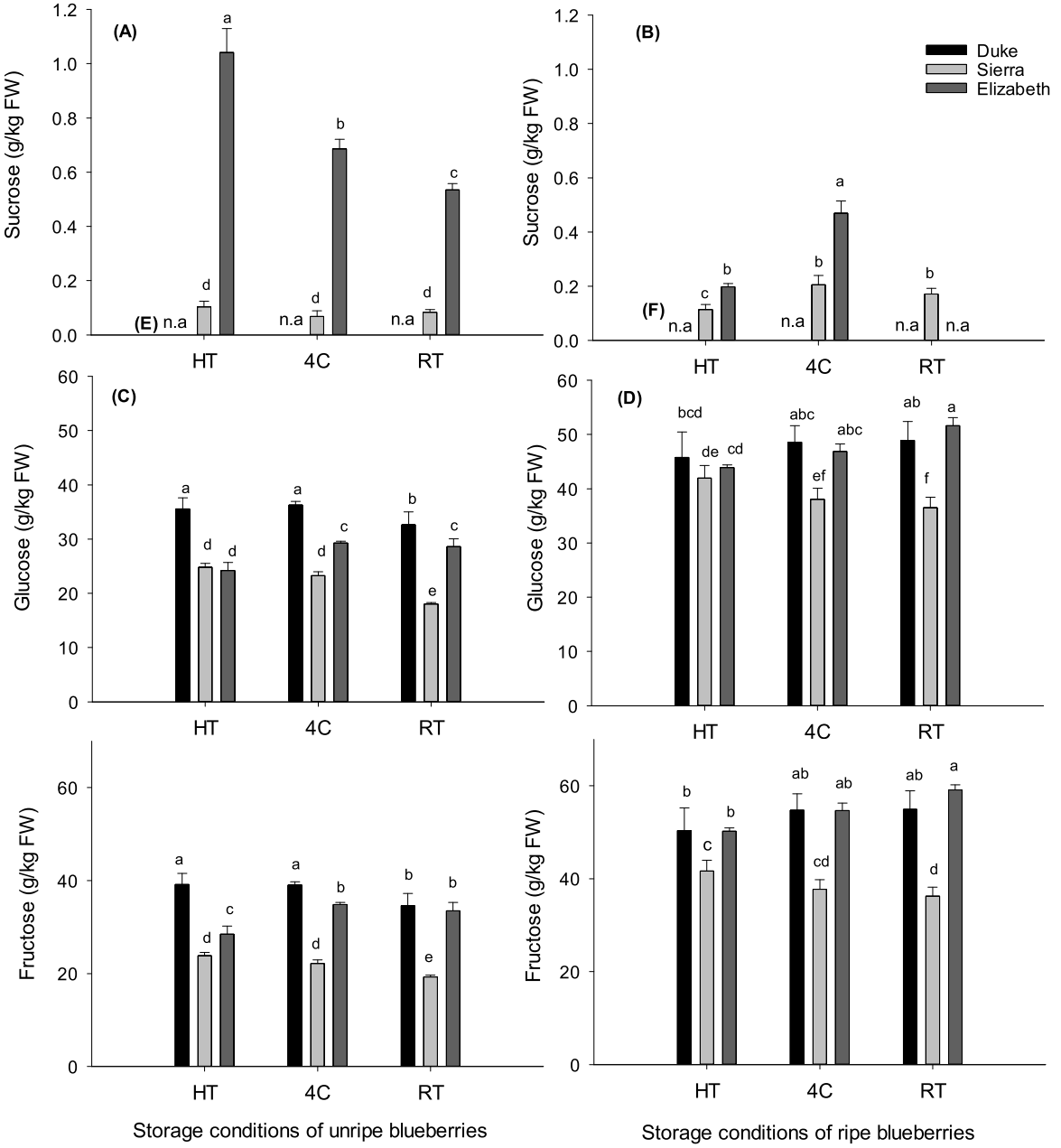

Fig. 2. Changes in the content of individual sugars (A, B) sucrose, (C, D) glucose, and (E, F) fructose in three blueberry fruit cultivars (Duke, Sierra, and Elizabeth) in un-ripe and ripe blueberries. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C, and RT for measuring after storing seven days at room temperature. "n.a" means not assayed. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

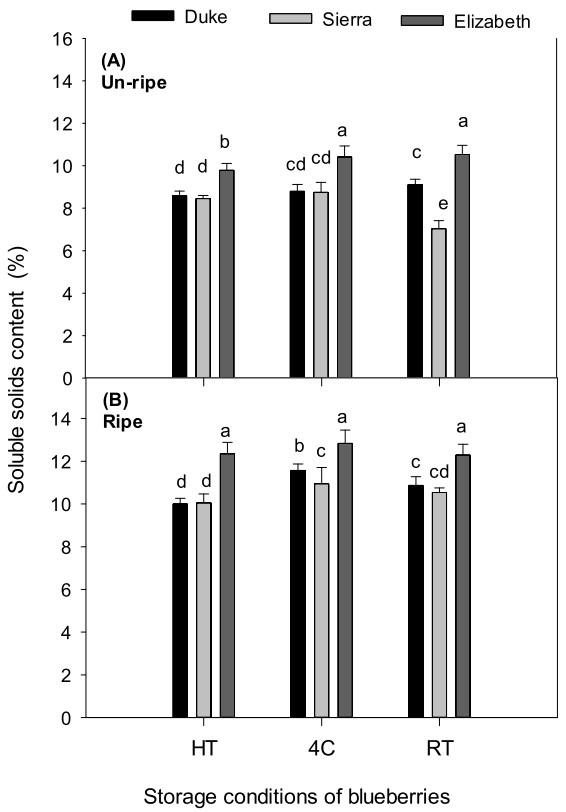

Fig. 3. Changes in soluble solids content in three blueberry fruit cultivars: (A) 'Duke' (B) 'Sierra' and (C) 'Elizabeth'. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

Elizabeth (Fig. 5C) after cold storage in both ripening stages. While *VcINV* expression significantly decreased from 1-fold to 0.5-fold in unripe fruit and from 1.1-fold to 0.7-fold in ripe Sierra fruit, it increased dramatically from 1-fold to 2.5-fold in unripe and from 1.9-fold to more than 2.9-fold in ripe Elizabeth fruit. Also, there was a slight increase in *VcINV* expression level in ripe Sierra samples at RT, reaching 1.9-fold. The expression level of *VcINV* was almost unchanged in Duke, except for a significant 8.2-fold increase in unripe fruit kept at RT (Fig. 5A). The expression levels of *VcSPS* increased in Duke and Elizabeth but not in Sierra when those fruits were mature (Fig. 6). Cold storage inhibited the expression levels of *VcSPS* in Duke and Sierra in both ripening periods, but it only induced the decrease of this transcript in the unripe Elizabeth (Fig. 6A, B). In Duke, cold storage inhibited the relative expression of *VcSPS* by 18% and 64% in unripe and ripe fruit, respectively. This transcript decreased to 0.16-fold and 0.20-fold in unripe and ripe fruit, respectively, in Sierra and declined to 0.59-fold in unripe Elizabeth at 4°C. The expression levels of *VcTMT* only reduced in Elizabeth when fruit was ripe (Fig. 7C). Compared to unripe fruit at HT (around 1-fold), storage conditions induced a significant decrease in the expression levels of *VcTMT* in Duke at both unripe (from 1 fold to approximately 0.1 fold) and ripe (from 1 fold to about 0.45 fold) (Fig. 7A). Still, they only caused a reduction of this transcript in unripe Elizabeth (from 1 fold to about 0.2 fold). Opposite, the gene expression levels of *VcTMT* increased at RT in Sierra (1.74-fold in ripe and 1.45-fold in unripe) (Fig. 7B). However, it reduced to 0.45-fold and 0.28-fold at 4°C in ripe and unripe

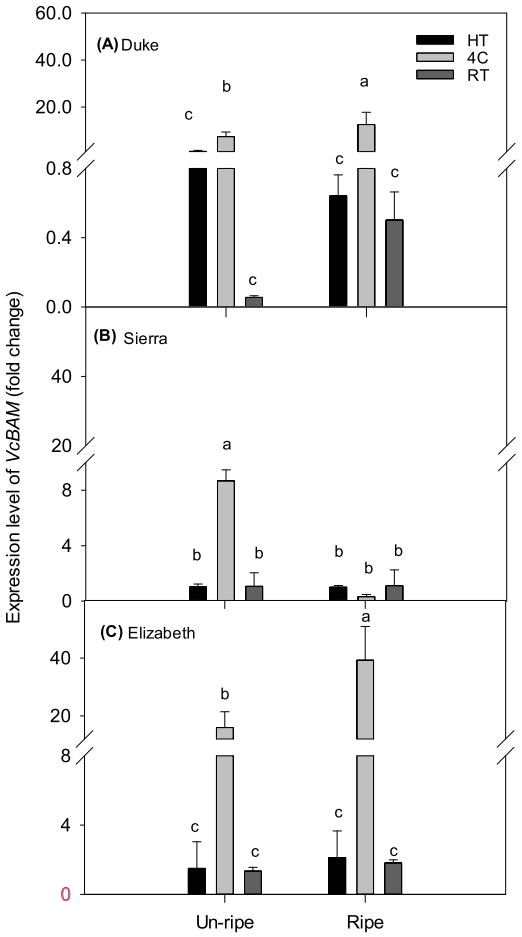

Fig. 4. Changes in the expression level of *VcBAM* (β amylase) at two ripening stages in three blueberry fruit cultivars: (A) 'Duke' (B) 'Sierra' and (C) 'Elizabeth'. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

Sierra. There were no significant differences between the two storage conditions for Duke and Elizabeth at both ripening stages (Fig. 7A, C).

3.5. Interactions between tagged genes and the metabolites of sugars and acids

The results of correlation analyses between the studied variables are shown in Fig. 8 (A, B). Glucose and fructose had a highly significant correlation regarding cultivar, ripening stage, and storage temperature (Fig. 8B). *VcBAM* had positive correlations with fructose and glucose in Elizabeth and Duke at 4°C storage but *VcBAM* had close interactions with these sugars in Sierra at 4°C in ripe blueberries (Fig. 8A, B). *VcSPS* had close interactions with *VcTMT* and negatively with quinic acid and citric acid. *VcINV* had a negative correlation with fructose in ripe Elizabeth fruit after storage at RT but a positive one with quinic acid in Duke at 4°C. *VcTMT* had a significant negative correlation with quinic acid in Sierra at RT but a positive one with glucose in unripe Duke at 4°C. It was

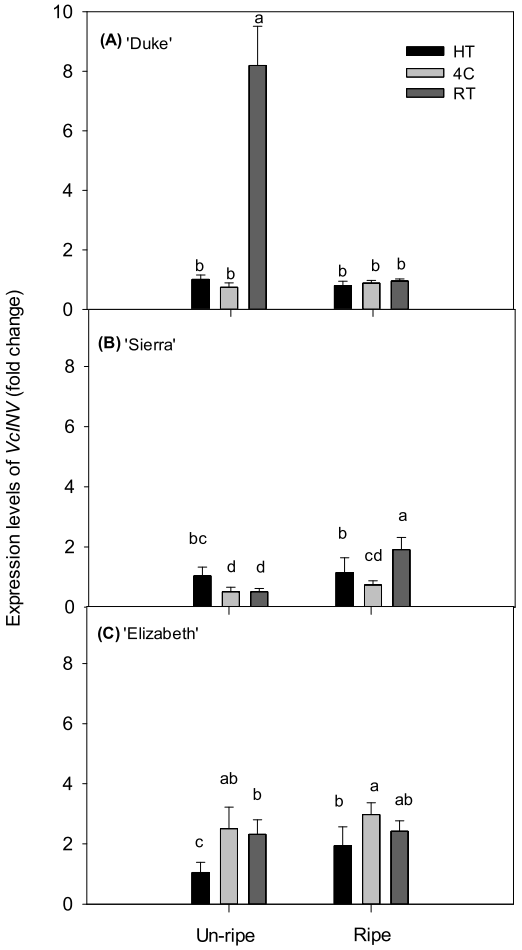

Fig. 5. Changes in the expression level of *VcINV (*invertase) at two ripening stages in three blueberry fruit cultivars: (A) 'Duke' (B) 'Sierra' and (C) 'Elizabeth'. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

interesting to see that *VcTMT* had close correlations with *VcBAM* and *VcBAM* was comparable to other tagged transcripts, SSC, glucose, and fructose.

4. Discussion

Organic acids are an essential source of respiratory energy in the plant cell. During the developmental stages of fruit, organic acids play a vital role in expanding cells by water uptake. They accumulate in the early stages of growing fruit, then decrease when the fruit is ripe [41]. Some previous studies investigated organic acid levels in blueberries [10, 12, 42, 43], and they found citric acid formed 77–87% of the organic acid content and quinic acid made up 4–11% of total acids in blueberries, with only a tiny amount of shikimic acid detected in the fruit [10]. This experiment noticed a high content of citric acid and a small amount of shikimic acid in all tagged cultivars. Many previous studies showed that the mechanism of organic acid accumulation came from the group

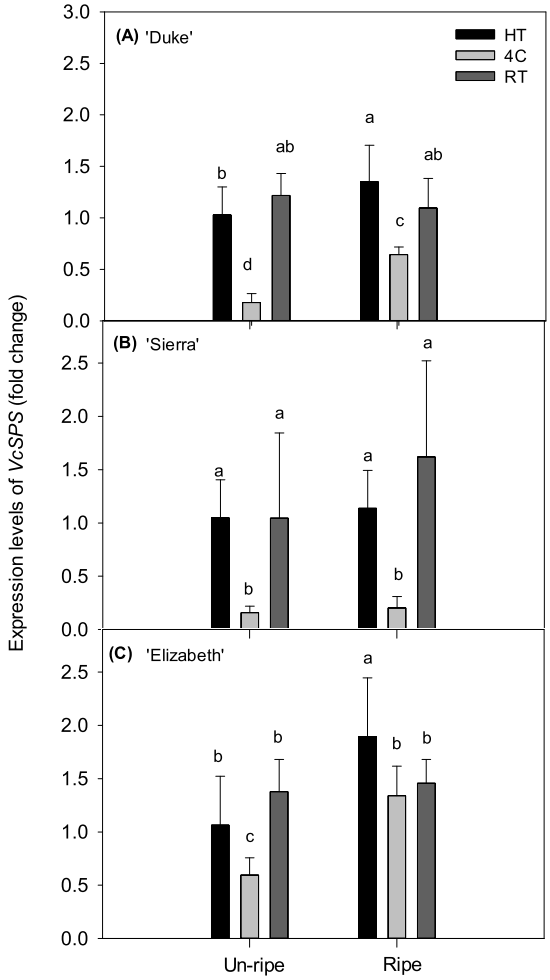

Fig. 6. Changes in the expression level of *VcSPS* (sucrose phosphate synthase) at two ripening stages in three blueberry fruit cultivars: (A) 'Duke' (B) 'Sierra' and (C) 'Elizabeth'. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

of metabolized reactions from the Krebs cycle [44, 45]. This processing continued to taking place in postharvest handling. In this study, storage conditions induced decreased citric acid and quinic acid in Duke and Elizabeth (but not in Sierra). A previous study reported that the phosphoenolpyruvate carboxykinase (PEP-c) played an essential role in the catabolism of organic acid in the flesh of blueberries [44]. The size of Duke and Elizabeth was larger than Sierra (data not showed). This phenomenon might be related to PEP carboxylase and carboxykinase activities, which led to changes in acid levels among tagged cultivars [46]. The higher quinic acid levels in Elizabeth at RT were the result of over-ripening. Thus, the different decreases in organic acids of blueberries depended on the size and cell expansion during the ripening stages of fruit. Storage conditions played a role as inhibitor or activator factors for these changes in the organic acid of blueberries.

In contrast to organic acids, the amounts of individual sugars increased with the ripening of fruit. Like previous studies [47–49], sucrose was not detected in Duke in any samples and disappeared after storage at RT in Elizabeth.

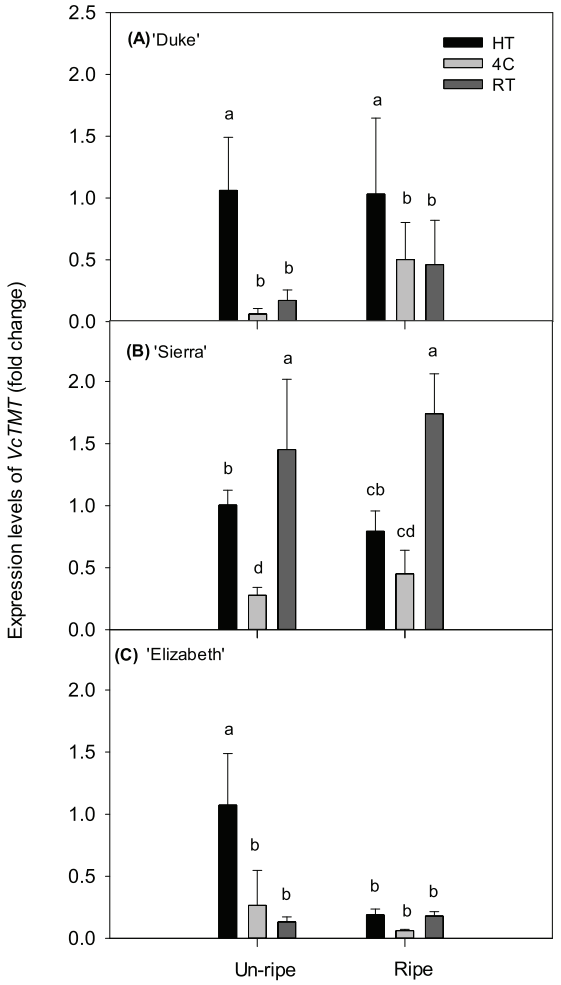

Fig. 7. Changes in the expression level of *VcTMT* (tonoplast monosaccharide transporter) at two ripening stages in three blueberry fruit cultivars: (A) 'Duke' (B) 'Sierra' and (C) 'Elizabeth'. The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The values are expressed as the means ± standard deviations of triplicate samples. Data in each figure with different letters are significantly different according to Duncan's multiple range tests at $p < 0.05$.

Sucrose is considered the primary carbohydrate in most plants. Sucrose depletion occurs through hydrolysis by the enzyme invertase, yielding glucose and fructose [50, 51]. Sucrose is an oligosaccharide (non-reducing sugar) and glucose and fructose are monosaccharides (reducing sugars). The levels of soluble sugars such as glucose, fructose, and sucrose determine the sweetness and quality of fruit [9, 52]. Glucose and fructose are the individual major sugars in blueberries and affect the flavor of fruit [4, 52]. After harvest, ripening affects the quality, sweetness, and taste of postharvest fruit. Glucose and fructose levels in postharvest blueberries also change depending on the ripening stage and cultivar [8]. The three cultivars in this study were from an organic farm, and organic blueberries tend to have significantly higher fructose and glucose levels [49]. The glucose and fructose analyses (combined with data not shown) showed that the sensory qualities were best in Elizabeth and worst in Sierra. Thus, when evaluating individual sugars, postharvest ripening and storage conditions are essential factors to consider.

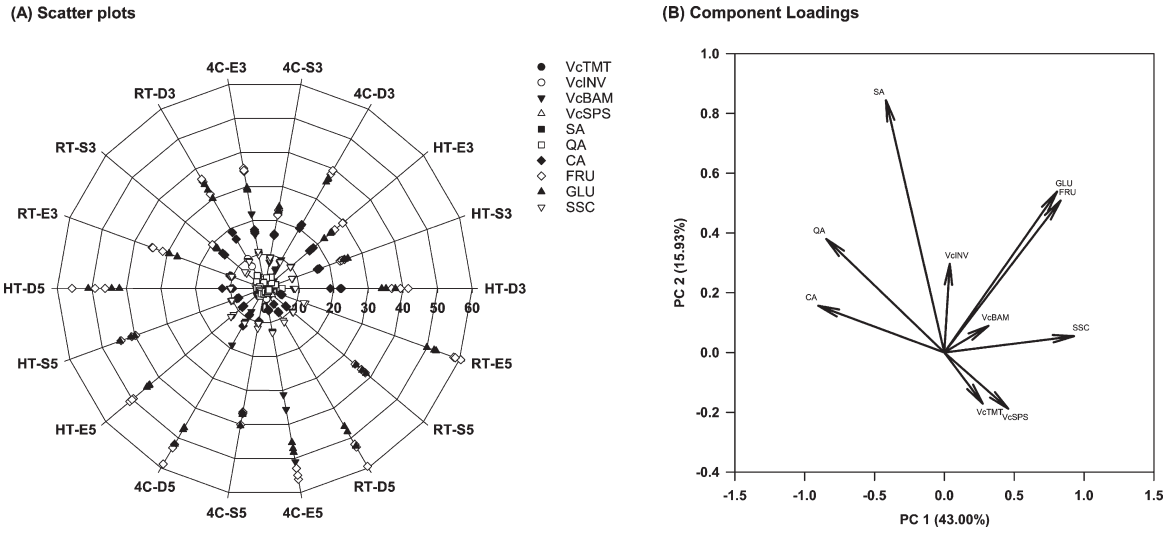

Fig. 8. Pearson correlation between metabolites and gene expression levels was expressed by (A) scatter plots and (B) component loadings. The interactions are calculated between gene expressions of *VcBAM*, *VcINV*, *VcSPS*, and *VcTMT* and metabolites of fructose (FRU), glucose (GLU), soluble solids content (SSC), and citric acid (CA), quinic acid (QA), and shikimic acid (SA). The storage conditions are denoted as HT for measuring at harvest time, 4C for measuring after storing seven days at 4°C; and RT for measuring after storing seven days at room temperature. The cultivars are denoted as D is Duke, S is Sierra, and E is Elizabeth. Number "3" showed ripening stage 3 (unripe fruit) and "5" showed ripening stage 5 (ripe fruit).

Changes in gene expression differed by cultivar, ripening stage, and storage temperature. β-amylase, a vital enzyme related to converting starch to maltose in plants, hydrolyzes the α-1,4-glucosidic bonds from the non-reducing end of the molecule, resulting in maltose formation. Although we cannot detect maltose in this study, a previous study demonstrated that the β-amylase gene (JQ911593) is affected by cold acclimation in Jersey and Sharp-blue blueberry cultivars. The authors reported an increase in β-amylase gene (*VcBMY*) expression linked to the reduction in starch and accumulation of solute sugars. During cold acclimation, *VcBMY* expression was 2-fold higher in Jersey than in Sharp-blue [19]. In a previous study, the expression of β-amylase family genes differed by developmental period and ripening stage and changed in response to abiotic stress in banana fruit [53]. *VcBAM* was upregulated under cold storage conditions in almost all samples (except ripe Sierra). It had close correlations with *VcTMT*, glucose, and fructose. This interaction showed a hypothesis that the transporter gene induced the activation of *VcBAM* and led to the increase of glucose and fructose. However, it might be due to starch-to-sugar conversion, although blueberry fruit has a low starch content (data not shown). This study did not show a significant correlation between *VcBAM* and sugars in Sierra, and the expression of *VcBAM* did not significantly increase in ripe Sierra fruit after harvesting. It was why the sugar metabolism did not happen in Sierra after harvesting.

Fruit quality also depends on invertase enzyme activity, which hydrolyzes sucrose into fructose and glucose, and plays a vital role in sugar metabolism in blueberry fruit. Shen et al.[54] investigated the function of acid invertase genes (AINV) in pepper fruit development. They found that AINV enzymes may be necessary for sucrose hydrolysis in the reproductive organs of pepper. Another study showed that the overexpression of *EjVIN* accelerates sucrose hydrolysis in loquat fruit [55]. Changes in invertase genes depend on the developmental and ripening periods of fruit. A recent study reported that high expression of sucrose invertase induces a decrease in glucose content in the early development stages of pumpkin [17]. However, in our research, *VcINV* decreased after harvest in unripe Sierra but increased in ripe Sierra and unripe Duke cultivars stored at RT, which might result in transglycosidation activity, transferring a hexose moiety to the primary alcohol group of mono- or

disaccharides [56]. Differences in the expression of *VcINV* between the two storage conditions considered herein depended on the cultivar. In the ripe fruit of Elizabeth, the activation of *VcINV* (MW383486) had an interaction with a decrease in fructose levels during RT storage, which resulted from over-ripening

Similar to invertase, sucrose phosphate synthase (SPS) helps determine the sucrose content in fruit [57]. Although the role of SPS has not been investigated in postharvest fruit, some studies have shown that gene expression of many SPS and TMT enzymes are only induced under cold conditions. Such genes include those associated with stress tolerance that encodes glycolytic and TCA cycle enzymes associated with protein synthesis machinery [58]. SPS (CUFF.14989.1) is related to glucose and fructose biosynthesis and is upregulated during the maturation of blueberries [8]. In our study, *VcSPS* (MT902322) was downregulated in cold storage but upregulated at ambient temperature. There was a high negative correlation between *VcSPS* and glucose and fructose in unripe Duke and Elizabeth fruit at RT and ripe fruit at 4°C. We hypothesized that the reduction of *VcSPS* led to an increase in fructose and glucose content. Cold storage inhibited the expression of *VcSPS* and induced the maintenance of fructose and glucose levels. This finding suggests that a mechanism that decreases sucrose synthase activity reduces growth, decreases starch, and reduces tolerance to anaerobic stresses in plants [59].

We designed the *VcTMT* (MT912540) transcript from sequences of the tonoplast monosaccharide transporter gene in the *Vaccinium* database. Using results about correlations between *VcTMT* and metabolites (glucose and quinic acid), combined with the expression levels of *VcTMT* under two storage conditions, we found that the increase in *VcTMT* induced the decrease in quinic acid and the increase in glucose in blueberries. *VvTMT2* (JX233818) expressed highly during the ripening and over-ripening of grape berries [60], which were similar results in the expression of *VcTMT* in Elizabeth. The tonoplast monosaccharide transporter (PbTMT4) participates in the vacuolar accumulation of sugars during the development of pear [21] and peach fruit [61]. In this study, storage conditions did not affect the expression of *VcTMT* in Duke and Elizabeth because of the characteristics of cells in the vacuoles of different fruit cultivars.

5. Conclusion

Ripening and storage conditions affected the synthesis of sugars and the degradation of organic acids and led to sweetness in blueberries. The metabolic mechanism was elucidated through the expression of tagged genes and analyses of metabolites. The activation of *VcBAM* and the inhibition of *VcSPS* and *VcINV* contributed to sugar biosynthesis, which was induced by the transporter *VcTMT*. The decrease of *VcTMT* when Elizabeth ripe supported the accumulation of sugar better than other cultivars. The inactivation of *VcBAM* also induced the metabolic process in Elizabeth. However, these genes did not induce significant changes in Sierra, which led to the sour taste in this cultivar, even after harvesting at full ripeness. The activation of *VcINV* correlated with a decrease in fructose content and higher quinic acid levels in Elizabeth at RT, resulting from over-ripening at RT. Thus, Elizabeth was the most relevant cultivar in ripening fruit under different postharvest conditions, then was Duke. Sierra was the least appropriate variety. This study provides insights into the mechanism underlying the ripening and maintenance of freshness and sweetness in postharvest blueberries. Our results promote a better understanding of suitable cultivars that can meet customer demands.

Conflict of interest

The authors have no conflict of interest to report

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Supplementary material

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References

[1] Yang S, Wang C, Li XY, Wu C, Liu C, Xue ZH, et al. Investigation on the biological activity of anthocyanins and polyphenols in blueberry. Journal of Food Science. 2021;86(2):614-27. 10.1111/1750-3841.15598

[2] Saftner R, Polashock J, Ehlenfeldt M, Vinyard B. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol Technol. 2008;49(1):19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

[3] Aires A, Carvalho R, Matos M, Carnide V, Silva AP, Goncalves B. Variation of chemical constituents, antioxidant activity, and endogenous plant hormones throughout different ripening stages of highbush blueberry (*Vaccinium corymbosum* L.) cultivars produced in centre of Portugal. J Food Biochem. 2017;41(6). https://doi.org/10.1111/jfbc.12414

[4] Gilbert JL, Guthart MJ, Gezan SA, de Carvalho MP, Schwieterman ML, Colquhoun TA, et al. Identifying breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses. Plos One. 2015;10(9). https://doi.org/10.1371/journal.pone.0138494

[5] Lin Y, Huang GH, Zhang Q, Wang YH, Dia VP, Meng XJ. Ripening affects the physicochemical properties, phytochemicals and antioxidant capacities of two blueberry cultivars. Postharvest BiolTechnol. 2020;162. https://doi.org/10.1016/j.postharvbio.2019.111097

[6] Paniagua AC, East AR, Heyes JA. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol Technol. 2014;95:50-9. https://doi.org/10.1016/j.postharvbio.2014.04.006

[7] Rosenfeld HJ, Meberg KR, Haffner K, Sundell HA. MAP of highbush blueberries: sensory quality in relation to storage temperature, film type and initial high oxygen atmosphere. Postharvest Biol Technol. 1999;16(1):27-36. https://doi.org/10.1016/s0925-5214(98)00102-1

[8] Li XB, Li CN, Sun J, Jackson A. Dynamic changes of enzymes involved in sugar and organic acid level modification during blueberry fruit maturation. Food Chem. 2020;309. https://doi.org/10.1016/j.foodchem.2019.125617

[9] Nookaraju A, Upadhyaya CP, Pandey SK, Young KE, Hong SJ, Park SK, et al. Molecular approaches for enhancing sweetness in fruits and vegetables. Sci Hort. 2010;127(1):1-15. https://doi.org/10.1016/j.scienta.2010.09.014

[10] Forney CFK, Wilhelmina | Jordan, Michael A. | Vinqvist-Tymchuk, Melinda R. | Fillmore, Sherry A.E. Blueberry and cranberry fruit composition during development. J Berry Res. 2012;2:169-77.

[11] Gibson L, Rupasinghe H, Forney CF, Eaton L. Characterization of changes in polyphenols, antioxidant capacity and physico-chemical parameters during lowbush blueberry fruit ripening. Antioxidants. 2013;2(4):216-29.

[12] Ayaz FA, Kadioglu A, Acar C, Turna I. Effect of fruit maturation on sugar and organic acid composition in two blueberries (*Vaccinium arctostaphylos* and *V-myrtillus*) native to Turkey. New Zealand J Crop Hort Sci. 2001;29(2):137-41. https://doi.org/10.1080/01140671.2001.9514171

[13] Blanch M, Goñi O, Sanchez-Ballesta MT, Escribano MI, Merodio C. Characterisation and functionality of fructo-oligosaccharides affecting water status of strawberry fruit (*Fragraria vesca* cv. *Mara de Bois*) during postharvest storage. Food Chem. 2012;134(2):912-9. https://doi.org/10.1016/j.foodchem.2012.02.203

[14] Hertog MLATM, Tijskens LMM, Hak PS. The effects of temperature and senescence on the accumulation of reducing sugars during storage of potato (*Solanum tuberosum* L.) tubers: A mathematical model. Postharvest Biol Technol. 1997;10(1):67-79. https://doi.org/10.1016/S0925-5214(97)87276-6

[15] Sweetman C, Deluc LG, Cramer GR, Ford CM, Soole KL. Regulation of malate metabolism in grape berry and other developing fruits. Phytochemistry. 2009;70(11):1329-44. https://doi.org/10.1016/j.phytochem.2009.08.006

[16] Miron D, Schaffer AA. Sucrose phosphate synthase, sucrose synthase, and invertase activities in developing fruit of *Lycopersicon-esculentum* Mill and the sucrose accumulating *Lycopersicon-hirsutum* Humb and Bonpl Plant Physiol. 1991;95(2):623-7. https://doi.org/10.1104/pp.95.2.623

[17] Wang CJ, Wang YL, Wang MM, Han HY, Luo YS, Ding WQ, et al. Soluble sugars accumulation and related gene expression during fruit development in Cucurbita maxima Duchesne. Sci Hortic. 2020;272:7. http://doi.org/10.1016/j.scienta.2020.109520

[18] Zhu Z, Liu RL, Li BQ, Tian SP. Characterisation of genes encoding key enzymes involved in sugar metabolism of apple fruit in controlled atmosphere storage. Food Chemistry. 2013;141(4):3323-8. http://doi.org/10.1016/j.foodchem.2013.06.025

[19] Lee JH, Yu DJ, Kim SJ, Choi D, Lee HJ. Intraspecies differences in cold hardiness, carbohydrate content and β-amylase gene expression of *Vaccinium corymbosum* during cold acclimation and deacclimation. Tree Physiol. 2012;32(12):1533-40.

[20] Ma QJ, Sun MH, Lu J, Liu YJ, Hu DG, Hao YJ. Transcription factor AREB2 is involved in soluble sugar accumulation by activating sugar transporter and amylase genes. Plant Physiol. 2017;174(4):2348-62. https://doi.org/10.1104/pp.17.00502

[21] Cheng R, Cheng Y, Lü J, Chen J, Wang Y, Zhang S, et al. The gene PbTMT4 from pear (*Pyrus bretschneideri*) mediates vacuolar sugar transport and strongly affects sugar accumulation in fruit. Physiol Plant. 2018;164(3):307-19. https://doi.org/10.1111/ppl.12742

[22] Reuscher S, Akiyama M, Yasuda T, Makino H, Aoki K, Shibata D, et al. The sugar transporter inventory of tomato: Genome-wide identification and expression analysis. Plant Cell Physiol. 2014;55(6):1123-41. https://doi.org/10.1093/pcp/pcu052

[23] del Olmo I, Blanch M, Romero I, Vazquez-Hernandez M, Sanchez-Ballesta MT, Escribano MI, et al. Involvement of oligosaccharides and sucrose-related genes on sucrose retention in strawberries from ripening to shelf-life. Postharvest Biology and Technology. 2020;169. http://doi.org/10.1016/j.postharvbio.2020.111301

[24] Itai A, Hatanaka R, Irie H, Murayama H. Effects of Storage Temperature on Fruit Quality and Expression of Sucrose Phosphate Synthase and Acid Invertase Genes in Japanese Pear. Horticulture Journal. 2015;84(3):227-32. http://doi.org/10.2503/hortj.MI-047

[25] Wu ZF, Tu MM, Yang XP, Xu JH, Yu ZF. Effect of cutting and storage temperature on sucrose and organic acids metabolism in postharvest melon fruit. Postharvest Biology and Technology. 2020;161. http://doi.org/10.1016/j.postharvbio.2019.111081

[26] Falagan N, Miclo T, Terry LA. Graduated controlled atmosphere: A novel approach to increase "Duke" blueberry storage life. Front Plant Sci. 2020;11. https://doi.org/10.3389/fpls.2020.00221

[27] Fan L, Forney C, Jordan M, Hildebrand P, Kalt W, Ryan D, et al., editors. Effect of ozone treatment and controlled atmosphere storage on quality and phytochemicals in highbush blueberries. VIII International Controlled Atmosphere Research Conference 600; 2001.

[28] Rodriguez J, Zoffoli JP. Effect of sulfur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol Technol. 2016;117:230-8. https://doi.org/10.1016/j.postharvbio.2016.03.008

[29] Hancock J. Blueberry characteristics range among the varieties. The Fruit Growers News, May Issue. 2001:36-7.

[30] Bal JJ, Balkhoven J, Peppelman G, editors. Results of testing highbush blueberry cultivars in The Netherlands. VIII International Symposium on Vaccinium Culture 715; 2004.

[31] Nunez-Barrios A, Nesmith DS, Chinnan M, Prussia SE. Dynamics of rabbiteye blueberry fruit quality in response to harvest method and postharvest handling temperature. Small Fruits Review. 2005;4(2):73-81. https://doi.org/10.1300/J301v04n02_08

[32] Boyette M, Estes E, Mainland C, Cline W. Postharvest cooling and handling of blueberries. AG (USA). 1993.

[33] Lobos GA, Callow P, Hancock JF. The effect of delaying harvest date on fruit quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum* L.). Postharvest Biol Technol. 2014;87:133-9. https://doi.org/10.1016/j.postharvbio.2013.08.001

[34] Zorenc Z, Veberic R, Stampar F, Koron D, Mikulic-Petkovsek M. Changeszin berry quality of northern highbush blueberry *(Vaccinium corymbosum* L.) during the harvest season. Turkish J Agric Forest. 2016;40(6):855-64. https://doi.org/10.3906/tar-1607-57

[35] Akagic A, Oras AV, Zuljevic SO, Spaho N, Drkenda P, Bijedic A, et al. Geographic variability of sugars and organic acids in selected wild fruit species. Foods. 2020;9(4):14. https://doi.org/10.3390/foods9040462

[36] Nguyen CTT, Kim J, Yoo KS, Lim S, Lee EJ. Effect of Prestorage UV-A, -B, and -C Radiation on Fruit Quality and Anthocyanin of 'Duke' Blueberries during Cold Storage. Journal of Agricultural and Food Chemistry. 2014;62(50):12144-51. 10.1021/jf504366x

[37] Nguyen TTC. Anthocyanin accumulation mechanism in highbush blueberry fruit by UV radiation: Seoul National University, Ph.D. Thesis; 2017.

[38] Nguyen H, Nguyen CTT, Jung C, Cheong JJ. AtMYB44 suppresses transcription of the late embryogenesis abundant protein gene AtLEA4-5. Biochem Biophysic Res Comm. 2019;511(4):931-4. https://doi.org/10.1016/j.bbrc.2019.03.006

[39] Nguyen CTT, Lim S, Lee JG, Lee EJ. *VcBBX*, *VcMYB21*, and *VcR2R3MYB* Transcription Factors Are Involved in UV B-Induced Anthocyanin Biosynthesis in the Peel of Harvested Blueberry Fruit. J Agric Food Chem. 2017;65(10):2066-73. https://doi.org/10.1021/acs.jafc.6b05253

[40] Nguyen CTT, Nguyen NH, Choi WS, Lee JH, Cheong, Jong-Joo Biosynthesis of essential oil compounds in *Ocimum tenuiflorum* is induced by abiotic stresses. J Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. 2020:1-8. https://doi.org/10.1080/11263504.2020.1857870

[41] Batista-Silva W, Nascimento VL, Medeiros DB, Nunes-Nesi A, Ribeiro DM, Zsogon A, et al. Modifications in Organic Acid Profiles During Fruit Development and Ripening: Correlation or Causation? Frontiers in Plant Science. 2018;9. http://doi.org/10.3389/fpls.2018.01689

[42] Correia S, Goncalves B, Aires A, Silva A, Ferreira L, Carvalho R, et al. Effect of harvest year and altitude on nutritional and biometric characteristics of blueberry cultivars. J Chem. 2016. https://doi.org/10.1155/2016/8648609

[43] Wang YF, Fong SK, Singh AP, Vorsa N, Johnson-Cicalese J. Variation of anthocyanins, proanthocyanidins, flavonols, and organic acids in cultivated and wild diploid blueberry species. Hortscience. 2019;54(3):576. https://doi.org/10.21273/hortsci13491-18

[44] Famiani F, Cultrera NGM, Battistelli A, Casulli V, Proietti P, Standardi A, et al. Phosphoenolpyruvate carboxykinase and its potential role in the catabolism of organic acids in the flesh of soft fruit during ripening. Journal of Experimental Botany. 2005;56(421):2959-69. http://doi.org/10.1093/jxb/eri293

[45] Vallarino JG, Osorio S. Organic acids. Postharvest physiology and biochemistry of fruits and vegetables: Elsevier; 2019. pp. 207-24.

[46] Tang N, Deng W, Hu N, Chen N, Li ZG. Metabolite and transcriptomic analysis reveals metabolic and regulatory features associated with Powell orange pulp deterioration during room temperature and cold storage. Postharvest Biology and Technology. 2016;112:75-86. http://doi.org/10.1016/j.postharvbio.2015.10.008

[47] Chea S, Yu DJ, Park J, Oh HD, Chung SW, Lee HJ. Preharvest beta-aminobutyric acid treatment alleviates postharvest deterioration of 'Bluecrop' highbush blueberry fruit during refrigerated storage. Sci Hort. 2019;246:95-103. https://doi.org/10.1016/j.scienta.2018.10.036

[48] Cordenunsi BR, Nascimento JRO, Lajolo FM. Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. Food Chem. 2003;83(2):167-73. https://doi.org/10.1016/S0308-8146(03)00059-1

[49] Wang SY, Chen CT, Sciarappa W, Wang CY, Camp MJ. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. J Agric Food Chem. 2008;56(14):5788-94. https://doi.org/10.1021/jf703775r

[50] Sturm A. Invertases, primary structures, functions, and roles in plant development and sucrose partitioning. Plant Physiol. 1999;121(1):1-7. https://doi.org/10.1104/pp.121.1.1

[51] Truernit E. Plant physiology: The importance of sucrose transporters. Curr Biol. 2001;11(5):169-71. https://doi.org/10.1016/s0960-9822(01)00085-9

[52] Gilbert JL, Olmstead JW, Colquhoun TA, Levin LA, Clark DG, Moskowitz HR. Consumer-assisted selection of blueberry fruit quality traits. HortSci. 2014;49(7):864-73. https://doi.org/10.21273/hortsci.49.7.864

[53] Miao HX, Sun PG, Miao YL, Liu JH, Zhang JB, Jia CH, et al. Genome-wide identification and expression analysis of the beta-amylase genes strongly associated with fruit development, ripening, and abiotic stress response in two banana cultivars. Front Agric Sci Engin. 2016;3(4):346-56. https://doi.org/10.15302/j-fase-2016127

[54] Shen L-B, Qin Y-L, Qi Z-Q, Niu Y, Liu Z-J, Liu W-X, et al. Genome-wide analysis, expression profile, and characterization of the acid invertase gene family in pepper. International journal of molecular sciences. 2019;20(1):15.

[55] Wang YP, Chen JW, Feng JJ, Qin QP, Huang JQ. Overexpression of a loquat (*Eriobotrya japonica* Lindl.) vacuolar invertase affects sucrose levels and growth. Plant Cell Tissue Organ Cult. 2015;123(1):99-108. http://doi.org/10.1007/s11240-015-0817-0

[56] Dewhirst RA, Fry SC. Oxalyltransferase, a plant cell-wall acyltransferase activity, transfers oxalate groups from ascorbate metabolites to carbohydrates. Plant Journal. 2018;95(4):743-57. http://doi.org/10.1111/tpj.13984

[57] Hubbard NL, Huber SC, Pharr DM. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing Muskmelon (*Cucumis*-Melo L) fruits. Plant Physiology. 1989;91(4):1527-34. http://doi.org/10.1104/pp.91.4.1527

[58] Dhanaraj AL, Alkharouf NW, Beard HS, Chouikha IB, Matthews BF, Wei H, et al. Major differences observed in transcript profiles of blueberry during cold acclimation under field and cold room conditions. Planta. 2007;225(3):735-51. https://doi.org/10.1007/s00425-006-0382-1

[59] Stein O, Granot D. An overview of sucrose synthases in plants. Front Plant Sci. 2019;10. https://doi.org/10.3389/fpls.2019.00095

[60] Çakir B, Giachino RR. VvTMT2 encodes a putative tonoplast monosaccharide transporter expressed during grape berry (*Vitis vinifera* cv. *Sultanine*) ripening. Plant Omics. 2012;5(6):576.

[61] Peng Q, Wang L, Ogutu C, Liu JJ, Liu L, Mollah MDA, et al. Functional analysis reveals the regulatory role of PpTST1 encoding tonoplast sugar transporter in sugar accumulation of peach fruit. Inter J Mol Sci. 2020;21(3). https://doi.org/10.3390/ijms21031112