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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Abscissic Acid Stimulates Softening and  
Pigmentation of ‘Jersey’ Highbush Blueberry  
Fruits during Ripening**

앱시스산에 의한 ‘저지’ 고관목 블루베리 과실의  
성숙 중 연화와 색소화 촉진

BY

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THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

# **Abscisic Acid Stimulates Softening and Pigmentation of ‘Jersey’ Highbush Blueberry Fruits during Ripening**

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## **ABSTRACT**

Ripening of some non-climacteric fruits such as grapes and strawberries is affected by abscisic acid (ABA). However, it is not clear whether this is a shared property in all non-climacteric fruits. Blueberries (*Vaccinium* spp.), known as non-climacteric fruits, accumulate high levels of anthocyanins during ripening. This alone makes blueberries a suitable tool to study the ripening of fruits. In this study, morphological and physiological changes were monitored in ‘Jersey’ highbush blueberries (*V. corymbosum*) during fruit development and effects of exogenous ABA on fruit ripening were investigated. To determine the effects of exogenous

ABA, fruits were treated with 10, 100, and 1,000 ppm ABA at 6 weeks after full bloom, before coloration initiated, and harvested at 0, 3, 6, 9, and 12 days after treatment (DAT). Fruit growth exhibited a double sigmoidal curve consisting of three distinguishable stages. Endogenous ABA contents significantly increased during fruit development. Anthocyanins increased dramatically at stage III, concurrently with the coloration. Among nine anthocyanins identified, malvidins were most abundant in the berries. All anthocyanins were strongly correlated with both a\* (redness) and b\* (blueness) values. ABA application at the onset of ripening accelerated the decrease of fruit firmness. Furthermore, a\* value increased and b\* value decreased more rapidly, indicating that exogenous ABA promoted coloration. Coinciding with the a\* and b\* values, all individual anthocyanins accumulated faster in ABA-treated fruits than in untreated fruits. These results indicate that ABA plays a crucial role in fruit softening and pigmentation of 'Jersey' highbush blueberries during ripening.

**Key words:** abscisic acid, anthocyanin, fruit coloration, fruit softening, highbush blueberry (*Vaccinium corymbosum*), non-climacteric fruit, ripening

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## INTRODUCTION

Fruits can be classified as climacteric and non-climacteric according to their ripening characteristics. Climacteric fruits, such as tomato, apple, banana, and peach, exhibit a burst of ethylene generation followed by a respiration increase at the onset of ripening (Giovannoni, 2001). These changes act as a leading signal for the initiation of ripening in all climacteric fruits.

In contrast to climacteric fruits, the hormonal control of ripening in non-climacteric fruits is largely unknown. Non-climacteric fruits do not show dramatic changes of ethylene or respiration, and ripening cannot be triggered by exogenous ethylene. Abscisic acid (ABA) has been suggested to play an important role in the ripening of some non-climacteric fruits, such as grape (Berli et al., 2011; Jeong et al., 2004; Peppi et al., 2008; Sandhu et al., 2011), strawberry (Jia et al., 2011; Li et al., 2011), and sweet cherry (Shen et al., 2014). However, it is not clear whether this is a shared property in all non-climacteric fruits.

Blueberry (*Vaccinium* spp.) fruits, known as non-climacteric, accumulate high levels of anthocyanins during ripening, which leads to a highly noticeable coloration process. This alone makes blueberries a suitable tool to study the ripening of non-climacteric fruits. However, the role of ABA in blueberry fruit ripening is obscure. This study was conducted to confirm the morphological and physiological changes of fruits during development in northern highbush blueberry

(*V. corymbosum*), and subsequently, to determine the effect of exogenous ABA on fruit ripening.

# LITERATURE REVIEW

## **Development of highbush blueberry fruits**

Growth of blueberry fruits exhibit a double sigmoid curve consisting of three stages, similarly to peach and grape (Darnell, 2006). At stage I, fruits go through rapid cell division and gain of weight. Stage II is a lag phase where little or no fruit growth occurs. Nevertheless, seed development is considered to be active in this period. Fruit grows rapidly again at stage III, in this case, by cell enlargement. Ripening, a process of fruits becoming more palatable, occurs at this stage. Clearly, the length of each stage depends largely on species, cultivar, and environment.

Blueberries are well known for their relatively high contents of anthocyanins which are efficient sources of dietary antioxidants (Bornsek et al., 2012). More importantly, anthocyanins are the main pigments responsible for the coloration of blueberry fruits. Among several hundred anthocyanins known to exist in nature, the predominant anthocyanins discovered in highbush blueberries are arabinosides, glucosides, and galactosides of cyanidin, delphinidin, malvidin, and petunidin (Kalt et al., 1999; Stevenson and Scalzo, 2012).

Fruit firmness changes dramatically during ripening. The fruit softens via the degradation of cell wall components, such as pectins, celluloses, and hemicelluloses (Proctor and Miesle, 1991). This results in a more desirable texture for seed

dispersers. As the fruit ripens and gets sweeter, concomitantly, sugar content increases and acidity decreases (Woodruff et al., 1960).

Whether blueberry fruits are climacteric or non-climacteric might still be a matter of debate. A rise in carbon dioxide and ethylene production was measured in highbush blueberries during stage III (Windus et al., 1976). However, no surge of carbon dioxide nor ethylene production was also reported during fruit development (Frenkel, 1972). In addition, exogenous ethylene did not induce a respiratory upsurge in highbush blueberries (Janes et al., 1978). Moreover, metabolism of ABA, an emerging key hormone in non-climacteric fruit ripening, correlated with anthocyanin accumulation in highbush blueberry fruits (Zifkin et al., 2012). Hence, highbush blueberry fruits are generally considered as non-climacteric.

### **Effects of exogenous ABA on non-climacteric fruit ripening**

Many aspects of fruit ripening, including coloration and softening, are important in terms of making the fruits more attractive and edible for frugivores including human. Therefore, understanding the mechanism of fruit ripening is essential to control the ripening process. In contrast to climacteric fruits, of which the ripening process is initiated and coordinated by ethylene, hormonal control of non-climacteric fruits is largely unknown. Recently, ABA has been considered to play a major role in non-climacteric fruit ripening (Leng et al., 2014).

Through proteomic approaches, Giribaldi et al. (2010) showed in ‘Cabernet

Sauvignon' grape, that exogenous ABA regulates mostly the same proteins known to be involved in ripening, such as vacuolar invertase 1, chalcone isomerase, and xyloglucan endotransglycosylase. Fruit ripening is also confirmed to be modulated at the molecular level, as exogenous ABA up-regulates ripening-related genes including those associated with cell wall modification and anthocyanin biosynthesis (Koyama et al., 2010).

ABA is known to be involved in the coloration of anthocyanin-accumulating fruits. Exogenous ABA increases anthocyanin content in grapes (Jeong et al., 2004; Koyama et al., 2010), strawberries (Jia et al., 2011; Li et al., 2014), and sweet cherry (Shen et al., 2014). Furthermore, ABA increases the expressions of anthocyanin biosynthesis genes in grape fruits, leading to the increase of anthocyanin accumulation (Jeong et al., 2004). On the other hand, silencing *NCED*, a gene encoding a key enzyme in ABA biosynthesis pathway, results in a colorless phenotype in sweet cherry (Shen et al., 2014). However, exogenous ABA does not affect anthocyanin content in southern highbush blueberries (*V. darrowii*; Buran et al., 2012).

Softening is accelerated by exogenous ABA in several non-climacteric fruits. In 'Pione' grapes, the decline of fruit firmness is promoted by exogenous ABA and delayed by ABA synthesis inhibitors, nordihydroguaiaretic acid (NDGA) and fluridone (Zhang et al., 2009b). Promotion of fruit softening by exogenous ABA is also confirmed in other grape cultivars, e.g., 'Redglobe' (Peppi et al., 2007), 'Flame

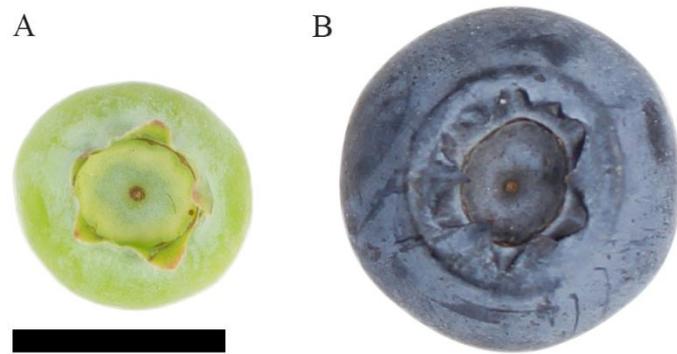
Seedless' (Peppi et al., 2006), and 'Benitaka' (Roberto et al., 2012), as well as in strawberries (Li et al., 2014).

## MATERIALS AND METHODS

### Plant materials and treatments

Ten-year-old highbush blueberry (*V. corymbosum* cv. Jersey) shrubs were grown at the experimental orchard of Seoul National University, Suwon, Republic of Korea. To investigate the morphological and physiological changes during fruit development, fruits from uniformly grown shrubs were harvested every week from 2 to 10 weeks after full bloom (WAFB).

ABA and an ABA biosynthesis inhibitor, NDGA, (Creelman et al., 1992) were treated in a randomized complete block design with three replications. (±)-ABA (Sigma-Aldrich, St. Louis, MO, USA) at 10, 100, and 1,000 ppm and NDGA (Sigma-Aldrich) at 30 ppm were used according to the methods of Jeong et al. (2004) and Zhang et al., (2009a), respectively. ABA and NDGA were dissolved in 5% (v/v) ethanol containing 0.1% (v/v) Tween 80. Control fruits were treated with 5% (v/v) ethanol containing 0.1% (v/v) Tween 80. At 6 WAFB, before the onset of fruit coloration (Fig. 1A), fruit clusters on shrubs were dipped into the solutions for 1 min. All treatments were conducted after sunset to avoid photodegradation of ABA. The fruit clusters were randomly sampled at 0, 3, 6, 9, and 12 days after treatment (DAT). Half of the fruits were used to determine length, diameter, weight, color, and firmness. The other half were immediately frozen in liquid nitrogen and



**Fig. 1.** ‘Jersey’ highbush blueberry fruits at 6 (A) and 10 (B) weeks after full bloom.

Bar = 10 mm.

stored at  $-80^{\circ}\text{C}$  for further determination of anthocyanins and ABA content.

### **Determination of fruit color and firmness**

Fruit skin colors were measured using a spectrophotometer (CM-2500d, Minolta Co., Osaka, Japan) and described by the CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space coordinates (Hunter and Harold, 1987). The  $L^*$  value represents the lightness of colors with a range from 0 to 100 (0, black; 100, white). The  $a^*$  and  $b^*$  values are ranged between  $-100$  and  $100$ . The  $a^*$  value is negative for green and positive for red. The  $b^*$  value is negative for blue and positive for yellow. For each fruit, the values were measured at two different points of the fruit equator.

Fruit firmness was analyzed using a CT3-4500 texture analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). Fruits were compressed using a flat probe of 2 mm diameter at a speed of  $0.5 \text{ mm s}^{-1}$ . The maximum peak force was recorded in  $\text{N}/\phi 2 \text{ mm}$ .

### **Identification of individual anthocyanins**

Anthocyanins were extracted according to the methods described by Gavrilova et al. (2011) with some modifications. Approximately 5 g of ground fruit tissues were extracted with 10 mL of a mixture containing acetone:acetic acid (99:1, v/v). The extracts were then sonicated for 15 min and centrifuged for 15 min at 3,000

rpm. The supernatants were evaporated until dryness using a rotary evaporator (EYELA N-1000S-W, Tokyo Rikakikai Co., Tokyo, Japan) at 37°C and then completely redissolved in 10 mL of 20% methanol. All samples were filtered through a 0.45 µm pore-size PTFE filter (Whatman Inc, Florham Park, NJ, USA).

Individual anthocyanins were identified using an HPLC-DAD system (Ultimate 3000, Thermo Dionex, Waltham, MA, USA) equipped with VDSpher PUR C-18 column (4.6 mm × 150 mm, 3.5 µm, VDS optilab, Berlin, Germany). Anthocyanins were eluted using a gradient of mobile phase A (aqueous 5% (v/v) formic acid) and mobile phase B (5% (v/v) formic acid in acetonitrile) in the following sequence: 0-30 min, 5-45% B; 30-35 min, 45% B; 35-36 min, 45-5% B; 36-40 min, 5% B. The flow rate was 0.8 mL min<sup>-1</sup> and detections were made at 520 nm. The amounts of anthocyanins were expressed as the external standard equivalent (malvidin 3-*O*-glucoside) from the calibration curve.

### **Determination of ABA content**

ABA was extracted as described by Forcat et al. (2008) with minor modifications. Freeze-dried fruit tissues were ground with an aid of liquid nitrogen. Approximately 200 mg of each samples in a 2 mL microfuge tube were resuspended with acetone:water:acetic acid (80:19:1, v/v/v) and powdered with two 3 mm beads using a TissueLyser II (Qiagen, Venlo, Netherlands) at 25 Hz for 3 min. The samples were stirred for 30 min, and vortexed and subsequently centrifuged at 13,000 rpm

for 2 min. The supernatant was lyophilized at room temperature, dissolved in 100  $\mu\text{L}$  of methanol:acetic acid (99:1, v/v), combined with 900  $\mu\text{L}$  of water:acetic acid (99:1, v/v), and centrifuged at 13,000 rpm for 1 min. The supernatant was then drawn through a  $\text{C}_{18}$  Sep-Pak cartridge (Waters Corp., Milford, MA, USA) (preconditioned first with 3 mL of methanol followed by 3 mL of 10% methanol containing 1% acetic acid) under vacuum. ABA was eluted from the cartridge using 1 mL of 80% methanol containing 1% acetic acid and then lyophilized at room temperature. The dried sample was resuspended in 200  $\mu\text{L}$  of 10% methanol, sonicated for 5 min, and then centrifuged at 13,000 rpm for 10 min.

ABA was identified in a 5600 Q-TOF LC/MS/MS system (AB Sciex, Foster City, CA, USA) using an Ultimate 3000 RSLC HPLC system (Thermo Dionex, Waltham, MA, USA), including a degasser, an auto-sampler, diode array detector (DAD), and a binary pump. LC separation was performed on a Hypersil GOLD column (2.1 mm  $\times$  50 mm, 1.9  $\mu\text{m}$ , Thermo Fisher Scientific, Waltham, MA, USA). ABA was eluted with a gradient of mobile phase A (aqueous 0.1% (v/v) formic acid) and mobile phase B (0.1% (v/v) formic acid in acetonitrile) in the following sequence: 0-3 min, 5% B; 3-11 min, 5-30% B; 11-14 min, 30-100% B; 14-16 min, 100% B. The flow rate was 0.25 mL  $\text{min}^{-1}$ . The auto-sampler was set at 4°C. The injection volume was 5  $\mu\text{L}$ . Mass spectra were acquired under negative electrospray ionization with an ion spray voltage of -4,500 V. The source temperature was 500°C. The curtain gas, ion source gas 1, and ion source gas 2 were 25, 50, and 50 psi,

respectively. Multiple reaction monitoring was used for quantitation of ABA ( $m/z$  263.1 to 153.1).

### **Statistical analysis**

Statistical differences were analyzed using SAS enterprise 4.3 (SAS Inst. Inc., Cary, NC, USA). Figures were generated using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

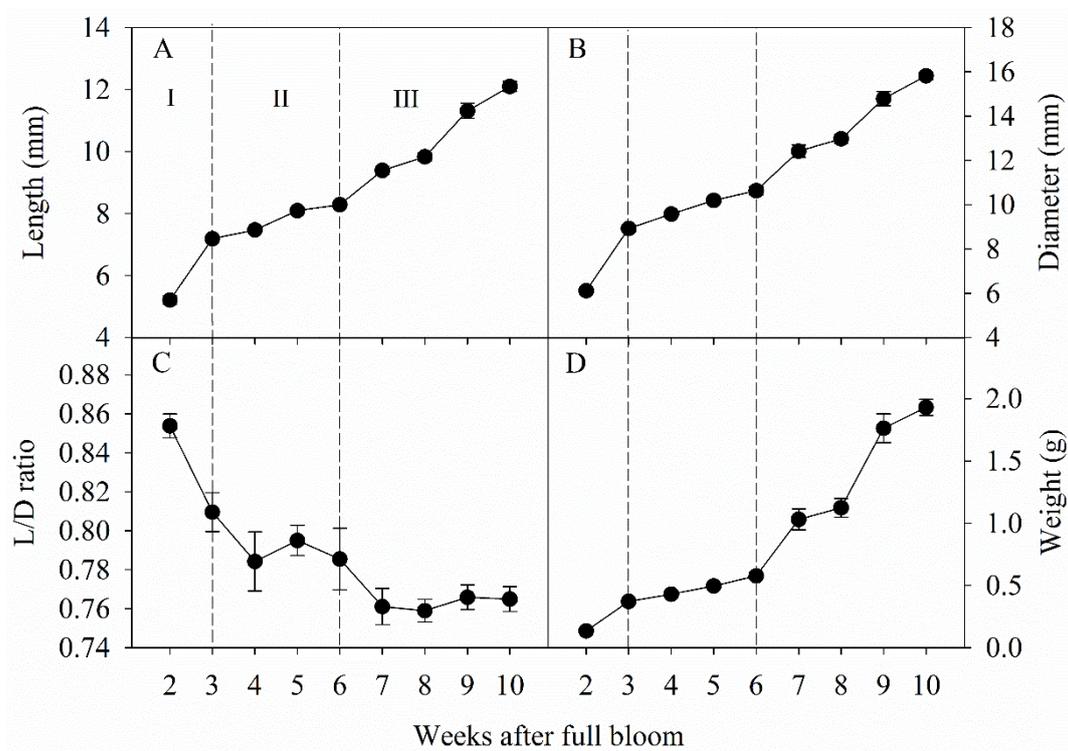
## RESULTS AND DISCUSSION

### **Morphological and physiological changes during fruit development**

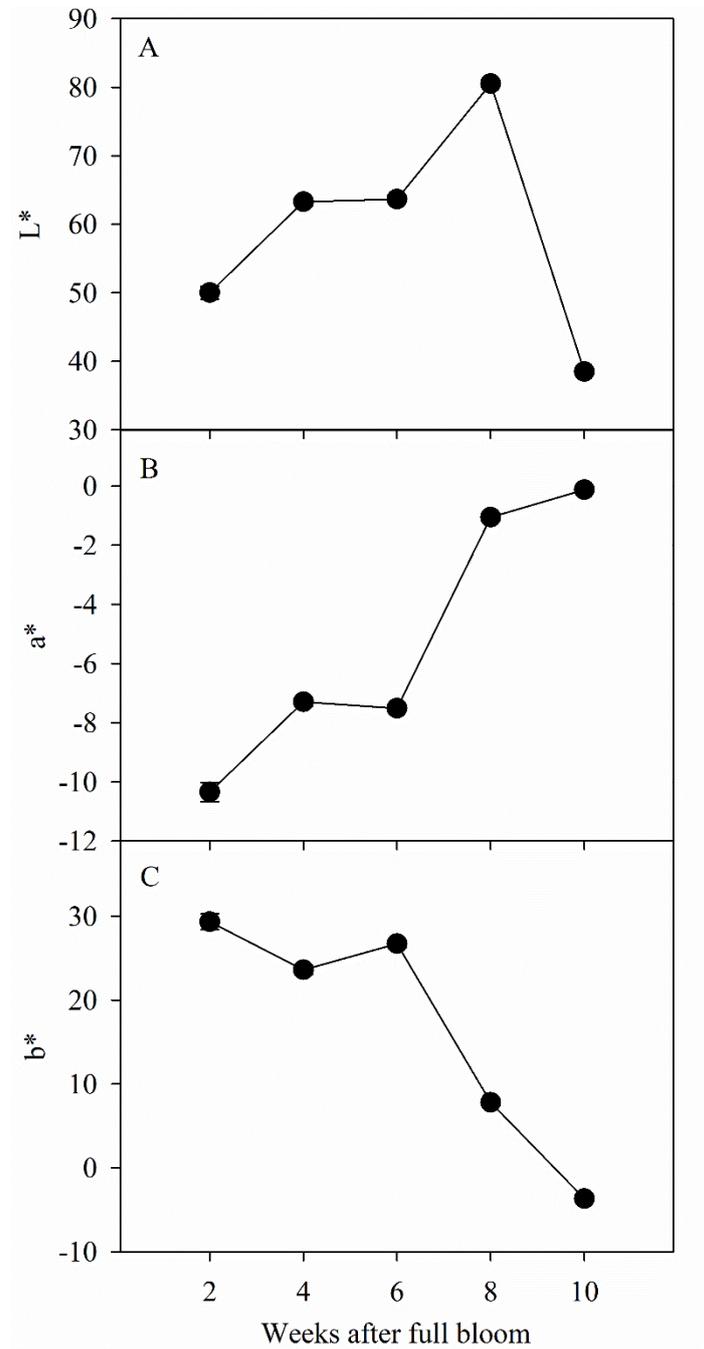
Fruit growth in 'Jersey' highbush blueberry followed a double sigmoid pattern which can be divided into three stages (Figs. 2A, B, D), as previously reported in various blueberry cultivars (Darnell, 2006). Fruit length, diameter, and weight rapidly increased during the initial 3 weeks of fruit development (stage I). After a 4-week period of lag phase (stage II), fruits enlarged rapidly again from 6 to 10 WAFB (stage III). Ratio of fruit length:diameter (L/D ratio) decreased until 7 WAFB, indicating that longitudinal growth preceded equatorial growth during fruit development (Fig. 2C). The final fruit shape was achieved at around 7 WAFB, when the L/D ratio started to remain constant.

Coloration of fruit skin was monitored biweekly in terms of CIE color space coordinates (Fig. 3).  $L^*$  value, representing lightness, increased from 2 to 8 WAFB, and then rapidly decreased at 10 WAFB (Fig. 3A). Change of skin color from yellowish green to purple was confirmed by the increase of  $a^*$  value and decrease of  $b^*$  value (Figs. 3B, C). Both  $a^*$  and  $b^*$  values changed substantially since 6 WAFB, implying the onset of coloration. This substantial change of color was concurrent with the rapid fruit enlargement at stage III (Fig. 2).

Individual anthocyanins responsible for the coloration of blueberry fruits were



**Fig. 2.** Morphological changes of 'Jersey' highbush blueberry fruit during development. Vertical bars indicate standard errors of the means ( $n = 30$ ). L/D ratio, ratio of fruit length:diameter.



**Fig. 3.** Changes of L\* (A), a\* (B), and b\* (C) values in ‘Jersey’ highbush blueberry fruit during development. Vertical bars indicate standard errors of the means (n = 30).

also determined biweekly. Identification of anthocyanins was based on comparison of UV-Vis and mass spectral data (Table 1) to previous work (Gavrilova et al., 2011). An anthocyanin characteristic HPLC-DAD profile is presented in Fig. 4. Nine anthocyanins were identified in ‘Jersey’ highbush blueberry fruits in the combination of three anthocyanidins (delphinidin and its *O*-methylated derivatives, petunidin and malvidin) and three glycosides (galactoside, glucoside, and arabinoside) (Table 1, Fig. 4). Until 6 WAFB, concentrations of all anthocyanins were very low or undetectable (Fig. 5). All anthocyanins largely increased at the period of 6 to 10 WAFB, coinciding with the coloration of fruit skin. At final harvest (10 WAFB), the predominant anthocyanins were malvidins, which are the most highly methylated form of delphinidin, followed by almost the equal amounts of delphinidins and petunidins.

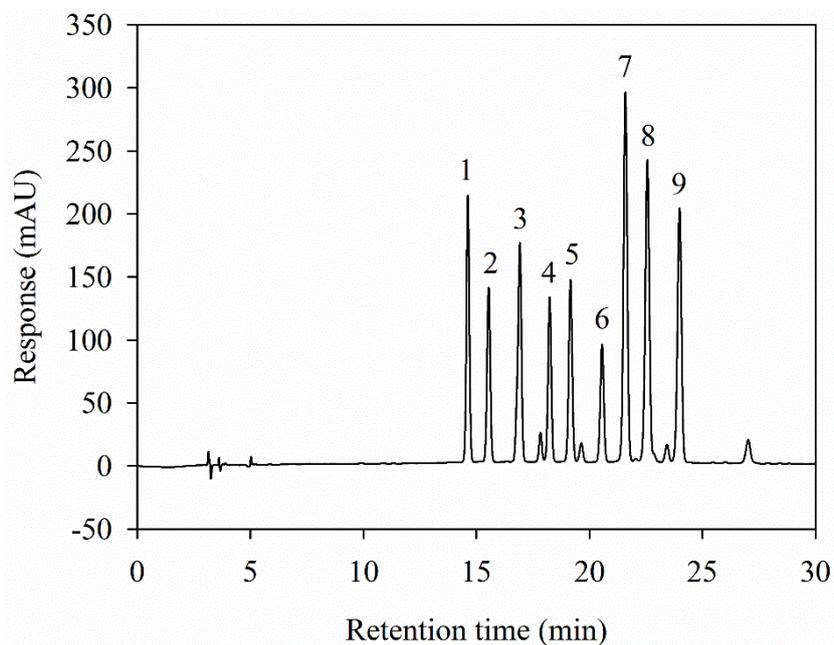
Strong correlations were found between individual anthocyanins and coloration (Table 2). All anthocyanins were highly correlated with both  $a^*$  and  $b^*$  values ( $P = 0.001$ ), showing positive correlations with  $a^*$  values (redness) and negative correlations with  $b^*$  values (blueness). However, individual anthocyanins were not correlated with  $L^*$  values, indicating that anthocyanins are responsible only for the purpleness, not for the lightness in ‘Jersey’ highbush blueberry fruits.

Using LC-MS/MS, ABA in ‘Jersey’ highbush blueberry fruit was quantified during development (Fig. 6). At initial development, ABA content decreased from 1.4 to 0.3  $\mu\text{g g}^{-1}$  dry weight (DW). Since 4 WAFB, however, ABA content increased

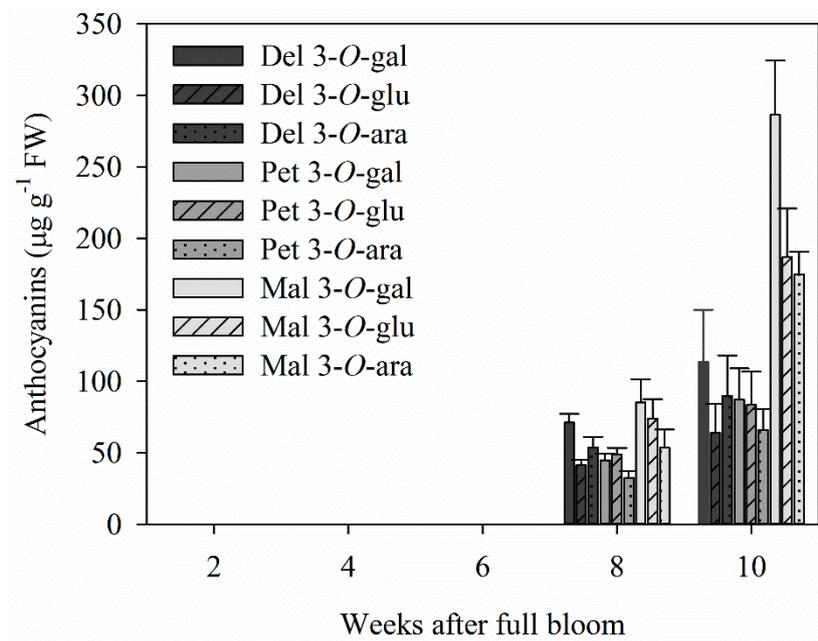
**Table 1.** Retention times, UV-Vis and mass spectral data of anthocyanins in ‘Jersey’ highbush blueberry fruits.

Peak	$t_R$ (min)	$\lambda_{max}$ (nm)	MS ( $m/z$ )	Anthocyanin
1	14.6	278, 523	465	delphinidin 3- <i>O</i> -galactoside
2	15.5	277, 523	465	delphinidin 3- <i>O</i> -glucoside
3	16.9	277, 524	435	delphinidin 3- <i>O</i> -arabinoside
4	18.2	278, 525	479	petunidin 3- <i>O</i> -galactoside
5	19.2	278, 524	479	petunidin 3- <i>O</i> -glucoside
6	20.6	277, 525	449	petunidin 3- <i>O</i> -arabinoside
7	21.6	278, 526	493	malvidin 3- <i>O</i> -galactoside
8	22.6	277, 527	493	malvidin 3- <i>O</i> -glucoside
9	24.0	278, 528	463	malvidin 3- <i>O</i> -arabinoside

Peak numbers and retention time ( $t_R$ ) correspond to Fig. 4.



**Fig. 4.** Typical HPLC-DAD chromatogram of anthocyanins in ‘Jersey’ highbush blueberry fruit monitored at 520 nm. Peak numbers correspond to 1, delphinidin 3-*O*-galactoside; 2, delphinidin 3-*O*-glucoside; 3, delphinidin 3-*O*-arabinoside; 4, petunidin 3-*O*-galactoside; 5, petunidin 3-*O*-glucoside; 6, petunidin 3-*O*-arabinoside; 7, malvidin 3-*O*-galactoside; 8, malvidin 3-*O*-glucoside; 9, malvidin 3-*O*-arabinoside.

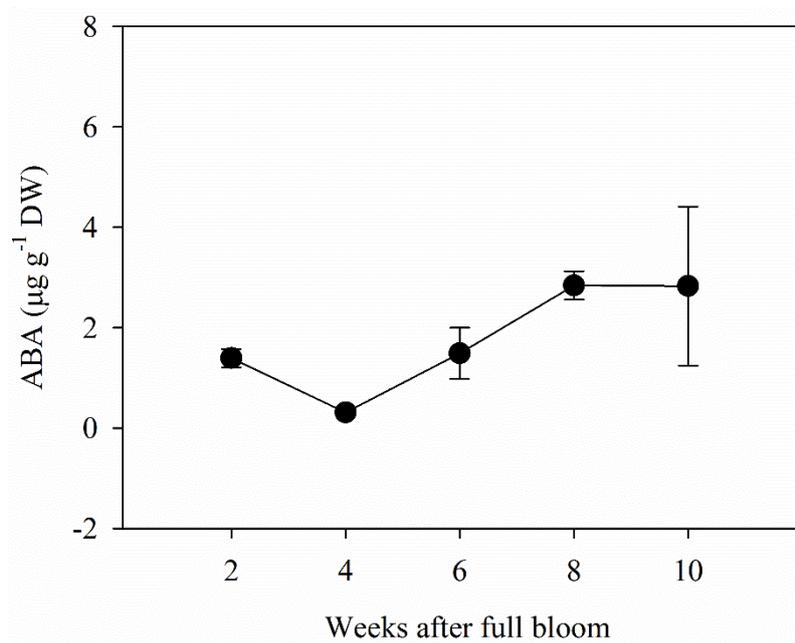


**Fig. 5.** Changes of individual endogenous anthocyanins in ‘Jersey’ highbush blueberry fruit during development. Vertical bars indicate standard errors of the means ( $n = 3$ ). Del, delphinidin; Pet, petunidin; Mal, malvidin; gal, galactoside; glu, glucoside; ara, arabinoside; FW, fresh weight.

**Table 2.** Pearson correlation coefficients ( $r$ ) between individual anthocyanins and CIE color space coordinates ( $L^*$ ,  $a^*$ , and  $b^*$ ) in ‘Jersey’ highbush blueberry fruits.

Anthocyanin	Pearson correlation coefficient ( $r$ )		
	$L^*$	$a^*$	$b^*$
Del 3- <i>O</i> -gal	-0.22 <sup>NS</sup>	0.84***	-0.89***
Del 3- <i>O</i> -glu	-0.21 <sup>NS</sup>	0.85***	-0.89***
Del 3- <i>O</i> -ara	-0.25 <sup>NS</sup>	0.84***	-0.89***
Pet 3- <i>O</i> -gal	-0.32 <sup>NS</sup>	0.85***	-0.91***
Pet 3- <i>O</i> -glu	-0.26 <sup>NS</sup>	0.85***	-0.90***
Pet 3- <i>O</i> -ara	-0.34 <sup>NS</sup>	0.85***	-0.92***
Mal 3- <i>O</i> -gal	-0.51 <sup>NS</sup>	0.81***	-0.92***
Mal 3- <i>O</i> -glu	-0.42 <sup>NS</sup>	0.83***	-0.92***
Mal 3- <i>O</i> -ara	-0.51 <sup>NS</sup>	0.82***	-0.93***

<sup>NS</sup>, \*\*\* Nonsignificant or significant at  $P = 0.001$ , respectively. Del, delphinidin; Pet, petunidin; Mal, malvidin; gal, galactoside; glu, glucoside; ara, arabinoside.



**Fig. 6.** Changes of endogenous abscisic acid (ABA) in 'Jersey' highbush blueberry fruit during development. Vertical bars indicate standard errors of the means (n = 3). DW, dry weight.

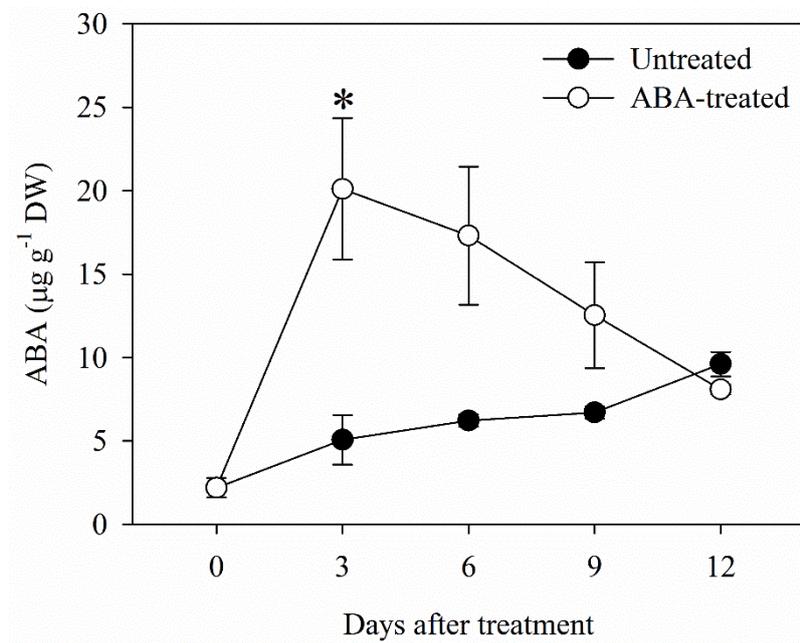
gradually, reaching the maximum concentration of approximately  $2.8 \mu\text{g g}^{-1}$  DW at 8 WAFB. Similar patterns were observed in 'Rubel' highbush blueberry (Zifkin et al., 2012), grape (Owen et al., 2009; Sun et al., 2010), and strawberry (Jia et al., 2011).

### **Effects of exogenous ABA on fruit growth and ripening**

To confirm the actual increase of internal ABA content when exogenous ABA was applied, changes of ABA content in untreated and ABA-treated (1,000 ppm) fruits were determined (Fig. 7). ABA content of untreated fruits increased steadily from  $2.2$  to  $9.6 \mu\text{g g}^{-1}$  DW during the 12-day period of observation. ABA-treated fruits exhibited maximal ABA content of  $20.1 \mu\text{g g}^{-1}$  DW at 3 DAT, which was significantly higher than that of untreated fruits ( $5.1 \mu\text{g g}^{-1}$  DW). Subsequently, ABA content in ABA-treated fruits gradually decreased, and finally, showed almost the same level as untreated fruits at 12 DAT.

Exogenous ABA at any concentrations had no obvious effects on fruit length, diameter, L/D ratio, and weight (Table 3). Although significant differences between untreated and ABA-treated fruits were observed in fruit length at 3 DAT when treated with 10 ppm ABA, no apparent patterns could be drawn. Therefore, these data suggest that ABA does not play a role in fruit growth at stage III.

On the other hand, exogenous ABA at 1,000 ppm reduced blueberry fruit



**Fig. 7.** Changes of abscisic acid (ABA) content in ‘Jersey’ highbush blueberry fruit treated without or with 1,000 ppm ABA. Asterisks denote significant differences using Dunnett’s test ( $P = 0.05$ ) and vertical bars indicate standard errors of the means ( $n = 3$ ). DW, dry weight.

**Table 3.** Effects of abscisic acid (ABA) application at various concentrations on fruit growth in ‘Jersey’ highbush blueberry.

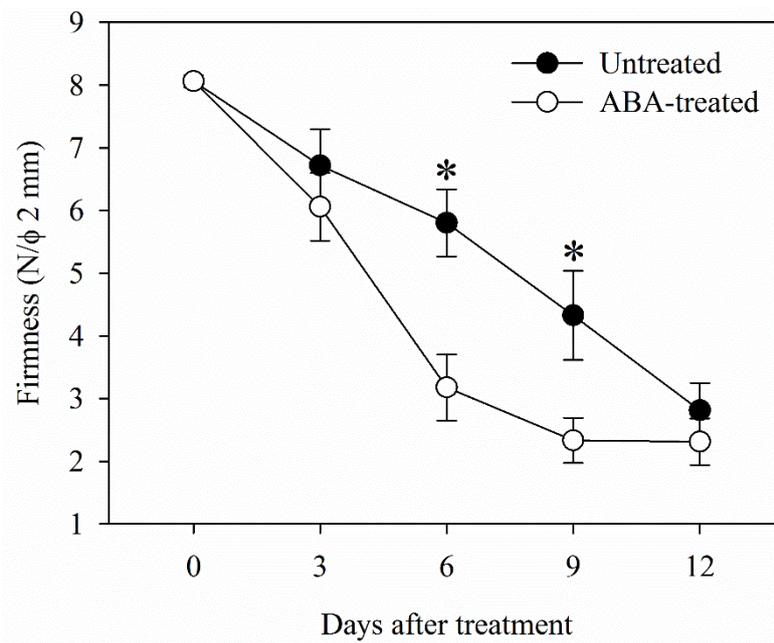
ABA (ppm)	DAT	Length	Diameter	L/D ratio	Weight (g)
		(mm)			
0	3	8.3 bB	11.3 abA	0.74 aB	0.72 abB
	6	9.1 abA	11.9 abA	0.77 aA	0.85 abB
	9	9.2 abA	11.9 abcA	0.78 abA	1.01 aAB
	12	9.7 aA	12.3 aA	0.78 aA	1.16 aA
10	3	9.2 aAB	11.5 aBC	0.80 aA	0.81 aBC
	6	8.9 bB	11.1 bC	0.81 aA	0.77 bC
	9	9.9 aA	12.7 abA	0.78 abA	1.11 aAB
	12	9.9 aAB	12.4 aAB	0.80 aA	1.18 aA
100	3	8.4 abB	11.0 abB	0.77 aA	0.68 abB
	6	9.1 abA	11.5 abAB	0.80 aA	0.83 abB
	9	9.1 bA	11.6 bcAB	0.79 abA	0.92 ab
	12	9.7 aA	12.4 aA	0.78 aA	1.20 aA
1,000	3	8.3 bB	10.9 abB	0.78 aAB	0.67 abB
	6	9.5 aA	12.4 aA	0.79 aAB	1.10 aA
	9	9.7 abA	12.8 aA	0.81 bB	1.15 aA
	12	9.8 aA	12.1 aA	0.81 aA	1.16 aA

Data are mean  $\pm$  standard error of means for n = 30. Significant differences are marked with lowercase letters (a, b, and c) within DAT and with uppercase letters (A, B, and C) within treatments using Tukey’s HSD test at  $P = 0.05$ . DAT, days after treatment; L/D ratio, ratio of fruit length:diameter.

firmness (Fig. 8), while ABA at 10 or 100 ppm did not (data not shown). Firmness of fruits treated with 1,000 ppm ABA was significantly lower at 6 and 9 DAT compared to that of untreated fruits, implying that ABA stimulates the softening process of blueberry fruits.

As in the case of fruit firmness, 1,000 ppm ABA promoted fruit coloration (Figs. 9, 10), whereas no significant differences were found when treated with 10 or 100 ppm ABA (data not shown). In fruits treated with 1,000 ppm ABA,  $a^*$  value was significantly higher and  $b^*$  value was significantly lower than that of untreated fruits, both at 6 DAT. Therefore, coloration of blueberry fruits towards purpleness was transiently hastened by exogenous ABA, since higher  $a^*$  value and lower  $b^*$  value represent redness and blueness, respectively.

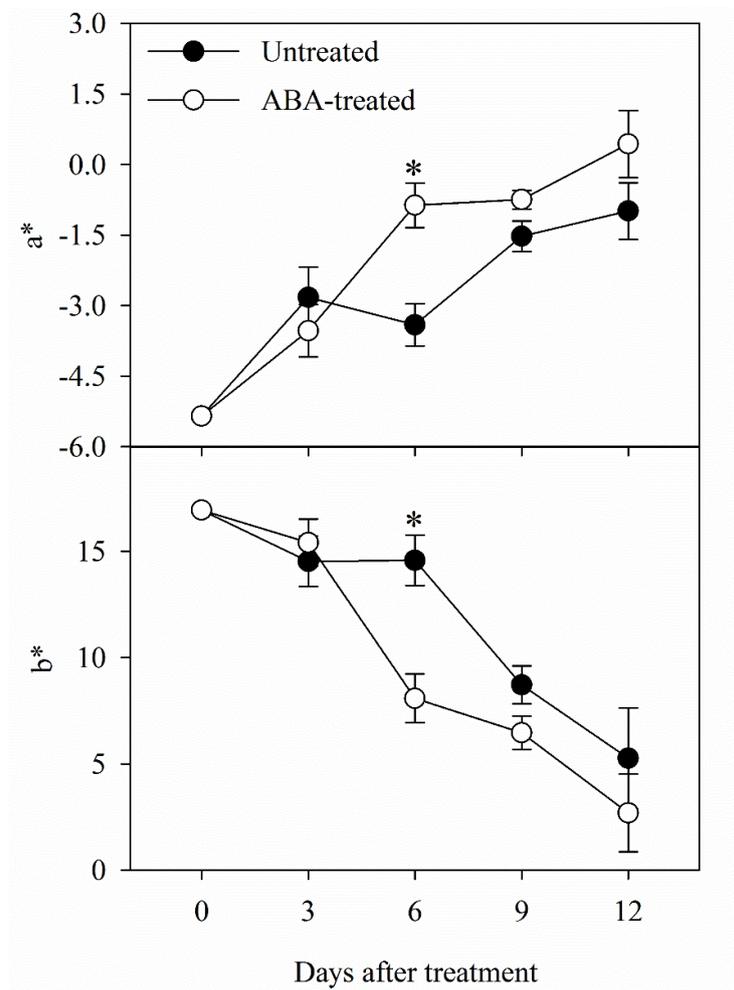
Individual anthocyanins, which were shown to be strongly correlated with blueberry fruit coloration (Table 2), were determined in untreated and ABA-treated fruits (Fig. 11). ABA was treated at 1,000 ppm to ensure its effect on fruit coloration. Overall, exogenous ABA significantly accelerated the accumulation of all nine anthocyanins. In particular, contents of delphinidins and petunidins showed significant differences at 9 DAT between untreated and ABA-treated fruits. The accumulation of malvidins, however, tended to be accelerated more slowly, since they are the latest to be biosynthesized among the three forms of anthocyanins (Kuhn et al., 2014). These observations are in agreement with studies of several other non-climacteric fruits, such as grapes (Jeong et al., 2004; Koyama et al., 2010),



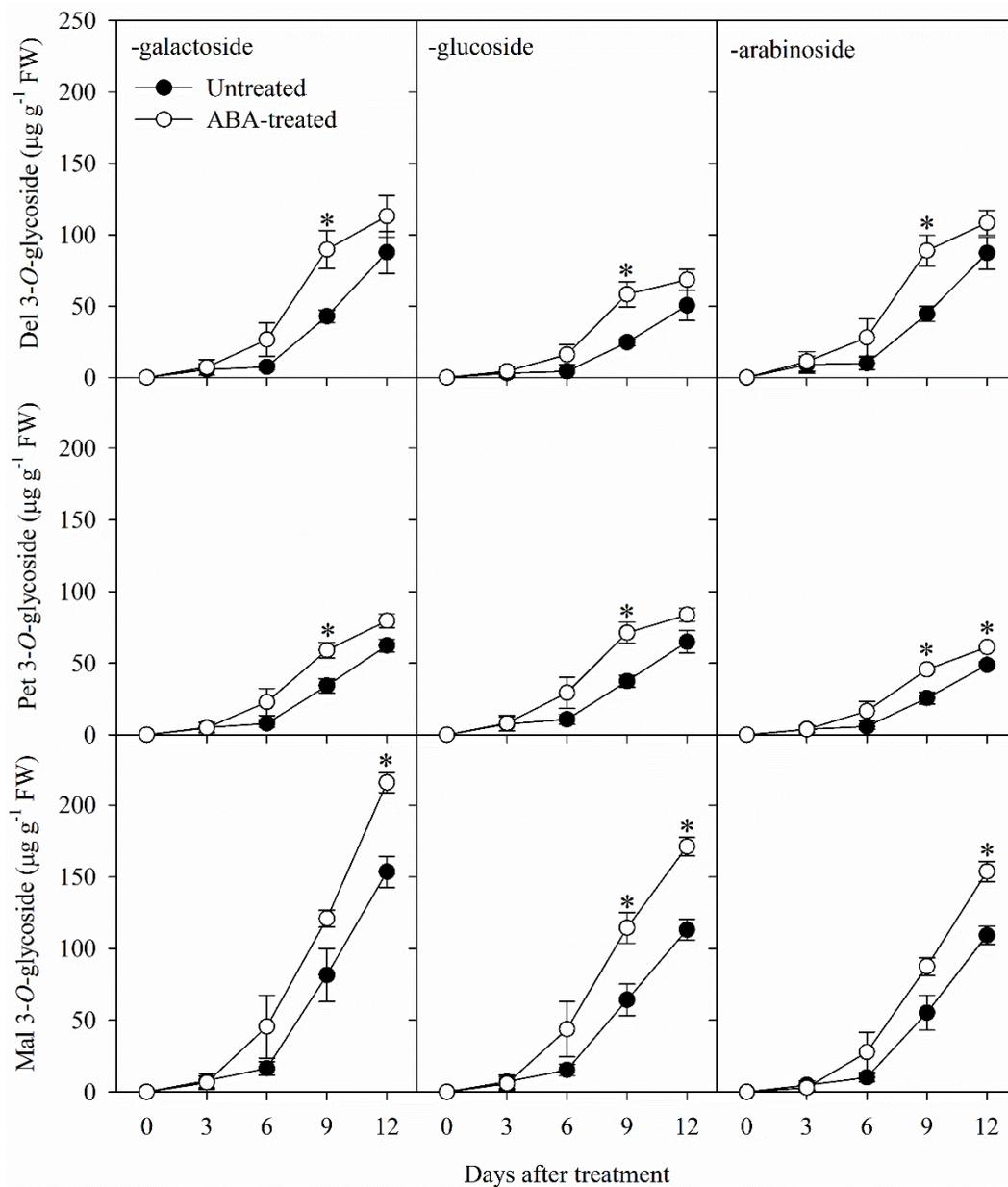
**Fig. 8.** Changes of firmness in ‘Jersey’ highbush blueberry fruit treated without or with 1,000 ppm abscisic acid (ABA). Asterisks denote significant differences using Dunnett’s test ( $P = 0.05$ ) and vertical bars indicate standard errors of the means ( $n = 30$ ).



**Fig. 9.** Fruit skin color of 'Jersey' highbush blueberry fruits treated without or with 1,000 ppm abscisic acid (ABA) at 6 days after treatment. Bar = 10 mm.



**Fig. 10.** Changes of  $a^*$  and  $b^*$  values in 'Jersey' highbush blueberry fruit treated without or with 1,000 ppm abscisic acid (ABA). Asterisks denote significant differences using Dunnett's test ( $P = 0.05$ ) and vertical bars indicate standard errors of the means ( $n = 30$ ).



**Fig. 11.** Changes of individual anthocyanins in ‘Jersey’ highbush blueberry fruit treated without or with 1,000 ppm abscisic acid (ABA). Asterisks denote significant differences using Dunnett’s test ( $P = 0.05$ ) and vertical bars indicate standard errors of the means ( $n = 3$ ). Del, delphinidin; Pet, petunidin; Mal, malvidin; FW, fresh weight.

strawberries (Jia et al., 2011; Li et al., 2014), and sweet cherry (Shen et al., 2014), showing promotive effects of exogenous ABA on the accumulation of anthocyanins. However, Buran et al. (2012) reported no significant effects of exogenous ABA on anthocyanin contents in southern highbush blueberry fruits (*V. darrowii*). This contradiction might be due to differing species of highbush blueberries and treatment methods employed.

NDGA has been reported to inhibit ABA production and thereby delay fruit ripening in grape (Zhang et al., 2009b) and sweet cherry (Shen et al., 2014). In this study, however, NDGA had no significant effects on fruit growth or ripening (data not shown), presumably due to insufficient concentration or inappropriate application timing. Since NDGA acts as an ABA biosynthesis inhibitor, not an ABA action inhibitor, NDGA should be applied before endogenous ABA starts to increase (Creelman et al., 1992). In the case of this study, the most appropriate timing of NDGA application would have been at around 4 WAFB, when ABA is almost absent in fruits (Fig. 6).

In conclusion, ABA stimulates the ripening of 'Jersey' highbush blueberry fruit which is non-climacteric by facilitating the decrease of fruit firmness and by accelerating anthocyanin accumulation responsible for coloration.

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## ABSTRACT IN KOREAN

포도나 딸기와 같은 일부 호홉 비급등형 과실의 성숙은 앱시스산 (abscisic acid, ABA)의 영향을 받는 것으로 알려져 있다. 그러나, 모든 호홉 비급등형 과실들이 이런 특성을 공유하는지는 명확하지 않다. 호홉 비급등형 과실로 알려진 블루베리(*Vaccinium spp.*)는 성숙 과정에서 높은 함량의 안토시아닌을 축적한다. 이런 점 때문에 블루베리는 과실의 성숙을 연구하기에 매우 적합한 도구가 될 수 있다. 이 연구에서는, ‘저지’ 하이부쉬 블루베리(*V. corymbosum*) 과실의 발달 기간 중 형태학적, 생리학적 변화를 관찰하고 외생 ABA가 과실 성숙에 미치는 영향을 조사하였다. 외생 ABA의 영향을 밝히기 위해, 과실의 착색이 시작되기 전인 만개 후 6주차에 10, 100, 그리고 1,000 ppm의 ABA를 과실에 처리하였고, 처리 후 0, 3, 6, 9, 그리고 12일차에 과실을 수확하였다. 발달 기간 중 과실의 생장은 세 개의 뚜렷한 단계들로 구분되는 이중 S자형 곡선을 따랐다. 과실 내의 내생 ABA 함량은 발달 과정 중 유의하게 증가하였다. 한편, 안토시아닌 함량은 단계 III에서 급격하게 증가하였으며, 이는 과실의 착색 시기와 일치하였다. ‘저지’ 하이부쉬 블루베리 과실에서 발견된 아홉 종류의 안토시아닌 중에서 말비딘 계열의 안토시아닌이 가장

함량이 높았다. 모든 안토시아닌은 a\*(붉음)와 b\*(푸름) 값 모두와 강한 상관성을 보였다. 과실 성숙 시작 시점에 외생적으로 ABA를 처리한 결과, 과실의 경도가 더 빨리 감소하였다. 더 나아가, a\* 값이 더 빨리 증가하고 b\* 값이 더 빨리 감소한 것으로 보아 외생 ABA가 착색을 촉진시켰다는 것을 알 수 있었다. 동시에 안토시아닌 또한 ABA를 처리한 과실에서 더 빨리 축적되었다. 이런 결과들은 ABA가 ‘저지’ 하이부쉬 블루베리 과실의 성숙 과정에서 과실의 연화와 색소화에 중대한 영향을 끼친다는 것을 나타낸다.