International Food Research Journal 21(3): 1131-1135 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Fruit developmental changes in abscisic and jasmonic acid contents of dragon fruit (*Hylocereous undatus*)

[1]Kammapana, L., [1,2]Jitareerat, P., [1,2] Wongs-Aree, C., [3]Yamauchi, N., [4]Kondo, S. and [1,2*]Srilaong, V.

[1]*Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand*
[2]*Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10150, Thailand*
[3]*Faculty of Agriculture, Yamaguchi University , Yamaguchi 753-8515, Japan*
[4]*Graduate School of Horticulture, Chiba University , Chiba 271-8510, Japan*

Article history

Received: 5 November 2013
Received in revised form: 16 January 2014
Accepted: 18 January 2014

Keywords

Dragon fruit
Abscisic acid
Jasmonic acid
ACC
Sugar

Abstract

The changes in abscissic acid (ABA) and jasmonic acid (JA) contents during fruit development of dragon fruit (*Hylocereus undaatus*) were determined. Associated changes in respiration, 1-aminocyclopropane-1-carboxylic acid (ACC) contents, and sugar levels were also measured. ACC content and respiration rate were high at early fruit development stages and gradually decreased as the fruit matured and ripened. In the pulp, ABA content increased before the increase in glucose and fructose contents. JA contents in the peel and pulp were high at the immature stage and decreased toward maturation. The results indicate that dragon fruit is non-climacteric and that JA may have a role in cell division in the developing fruit while ABA may induce fruit maturation.

Introduction

Dragon fruit (*Hylocereous undatus*) is widely cultivated in Thailand and other tropical countries. The fruit has high antioxidant properties that could minimize the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. There is very little published information about dragon fruit physiology, which is important to the development of quality management strategies. Some reports revealed the changes in sugar content in dragon fruit after harvesting, such as that of Wichienchote *et al.* (2010), but to our knowledge, there is no report on the changes of physiologically active substances during fruit development and maturation.

Among plant growth regulators, abscisic acid (ABA) may play a vital role in maturation and ripening of non-climacteric fruit (Kondo and Gemma, 1993; Kondo and Kawai, 1998). ABA may do the same in dragon fruit which is non-climacteric type as its respiration rate does not exhibit a large peak at the onset of ripening; this is concomitant with low levels of ethylene production. Previous works have shown that 9-cis-epoxycarotenoid dioxygenase (NCED), an upstream enzyme in ABA biosynthesis pathway, is implicated in the ripening of several fruits including orange (*Citrus sinensis*) (Rodrigo *et al*., 2006), grape (*Vitis vinifera*) and peach (*Prunus persica*) (Zhang *et al.*, 2009). In addition, ABA was shown to promote sugar accumulation in strawberries (*Fragaria Anamosa*) (Jia *et al*., 2011) and peaches (*Prunus persica*) (Kobashi *et al.*, 2001). On the other hand, jasmonic acid (JA) was found to influence C_2H_4 production in apple (Saniewski *et al*., 1986). Kondo *et al*. (2000) showed that an-thocyanin synthesis in apple was induced by n-propyl dihydrojasmonate (PDJ) application. PDJ was also shown to stimulate callus formation in sweet cherry pulp discs (Kondo *et al.*, 2002). However, JA did not affect anthocyanin formation in sweet cherries (Kondo *et al*., 2002) and grape berries (Kondo and Fukuda, 2001). Therefore, JA effects may differ with fruit type. In non-climacteric sweet cherries, ABA content increased before maturation and then decreased toward harvest while JA content was high at the beginning of fruit development and decreased toward maturation (Kondo and Tomiyama, 1998; Kondo *et al.*, 1999). In the present study, the changes in JA and ABA contents during fruit development of dragon fruit were determined.

Material and Methods

Fruit samples

Nine randomly selected 10-year-old white-pulp dragon fruit trees in a local orchard at Pathumthani Province, Thailand, were used. Two fruits per tree were randomly harvested at 10, 20, 30 and 40 days after anthesis (DAA) in July, 2012. Fruit were selected

*Corresponding author.
Email: *varit.sri@kmutt.ac.th*

for uniformity in size and freedom from defects. After harvest, the 18 fruit samples were randomly divided into two groups. The first group was used for fruit weight and respiration measurement while the second group was used for chemical analysis using peel and pulp samples which were frozen in liquid N_2 and stored at -20ºC until analysis.

Respiration measurement

Three fruits were sealed in a 2.9 L plastic container for 3 h at 25 ± 2ºC. One mL of headspace gas was collected and injected onto a gas chromatograph (GC) (GC-8A; Shimadzu Scientific Instrument, Japan) equipped with a porous polymer column (Porapak Q; 3.0 mm i.d. x 2.0 m) and thermal conductivity detector (TCD) for CO_2 measurement. Each measurement was replicated three times.

ACC analysis

ACC content was analyzed by the method of Kondo *et al.* (2002) with slight modifications. Two gram pulp or peel samples from three fruits per replicate were homogenized in 10 mL of 0.1 M HCl. The homogenate was centrifuged at 15,000 ×g for 20 min at 4ºC. The supernatant was decanted, placed on ice, and used as a crude extract. The ACC content was assayed by mixing 2 mL of crude extract, 0.2 mL of 0.1M $HgCl_2$ and 0.2 mL of 5% NaClO/saturated NaOH 2:1 (v/v). Then, 2 mL of headspace gas was measured using a GC with flame ionization detector (FID) (GC 2014, Shimadzu, Kyoto, Japan) and 2.2 mm i.d. × 2.0 mm column (Porapak Q; Waters, Milford, MA), with column temperature of 50ºC and helium flow rate of 30 mL min^{-1}. ACC content was estimated from the standard curve.

ABA analysis

The extraction and quantification of endogenous ABA was performed following the method of Setha and Kondo (2009) with some modifications. One gram peel or pulp samples (three replications of three fruits) were homogenized in 20 mL of cold 80% methanol (v/v) with 200 ng 3',5',5',7',7',7'-hexadeuterated ABA (ABA-*d6*) as an internal standard. The homogenate was centrifuged, then filtered through filter paper (pore size 4 nm, KIRIYAMA Co. Ltd., Tokyo, Japan), and then evaporated to the aqueous phase. The aqueous residue was adjusted to pH 2.5 with 0.1 M hydrochloric acid and partitioned three times with 20 mL of ethyl acetate. The ethyl acetate extract was combined, evaporated to dryness, and redissolved three times in 1 mL of ethyl acetate. The solvent was dried, and the residue was redissolved in 1 mL of 4.8 M acetonitrile containing 20 mM acetic acid. The solution was filtered through a nitrocellulose filter (pore size 0.22 µm, EMD MILLPORE Co., Billerica, MA, USA) and fractionated by high performance liquid chromatography (HPLC; Spectroscopic, Tokyo, Japan) using an ODS Mightysil RP-18 column (4.6 mm i.d. × 250 mm) with a gradient of 4.8-9.6 M acetonitrile containing 20 mM acetic acid over a 30 min period at a flow rate of 1.3 mL min^{-1}, and detected by UV absorption at 254 nm. The fraction containing ABA and the internal standard was collected, evaporated to dryness, and re-dissolved three time in 0.5 mL of methanol and dried in vacuum. The residue was re-dissolved with 1 mL of 10% (v/v) methanol in diethyl ether and then methylated with diazomethane for 10 min. The methyl ether of ABA was quantified and identified by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM; model QP5000; Shimadzu, Kyoto, Japan) using an InertCap 1 MS column (GL Sciences, Tokyo, Japan; 0.25 mm i.d.).

JA analysis

JA content of peel and pulp was analyzed by the method of Kondo et al. (2005) with modifications. One gram peel or pulp samples (three replications of three fruits) was homogenized with 100 µL (±)-2-(2,3-2H2) JA as the internal standards in 10 mL of saturated NaCl solution and 20 mL of diethyl ether containing 0.005% butylated hydroxytoluene (BHT) as an antioxidant. The ether phase was removed after centrifugation at 8000 × g for 15 min. The aqueous layer was extracted a second time with 20 mL of diethyl ether containing 0.005% BHT. The pooled ether extract was dried under warm air. The residue was dissolved in 200 µL of chloroform/isopropylethylamine, 1:1 (v/v), and derivatized at 50ºC for 60 min with pentafluorobenzyl bromide (PFB). Analysis of PFB-JA was conducted using GC-mass spec-troscopy with selected ion monitoring [QP 5000; Shimadzu, Kyoto, Japan; 25 m × 0.25 mm i.d. column (CP-Sil 5 CB; Chrompack, Middelburg, The Netherlands)]. The column tempera-ture gradient was 60ºC for 2 min, 60 to 270 at 10ºC min^{-1}, and 270ºC for 35 min with a 50.2 cm s^{-1} linear He flow and 70 eV electron potential.

Analysis of sugar content

Ten gram pulp samples (three replications of three fruits) were placed in 100 mL of boiling 80% ethanol for 15 min, cooled and homogenized, filtered through a filter paper, and the solvent was evaporated in vacuo to the aqueous phase. The residues were re-dissolved with 50 mL of distilled water. 500 µL of crude extract was mixed with 500 µL of 100% ace-

tonitrile and passed through a 0.45 µm filter. 40 µL of solution was determined quantitatively for sugar content by the HPLC system (Hitachi, Tokyo, Japan; column = Sodex Asahipak NH2P-50 (Showa Denko Co, Tokyo, Japan), 4.6 mm I.D. × 30 cm; column temperature : 30 ºC; mobile phase : 78% acetonitrile; flow rate 1.0 mL•min^{-1}; detector : RI).

Statistical analysis

Results were analyzed using the SAS statistical program (SAS Institute, Cary, NC) , Mean separation was done by the Fisher's least significant difference ($P \leq 0.05$).

Results

Fruit weight, respiration and ACC content

Fruit weight continuously increased from 10 DAA until harvest at 40 DAA when the fruit ripened (Figure 1A). Respiration rate was highest at 10 DAA and decreased gradually until harvest (Figure 1A). On the other hand, ACC content in the pulp was highest at 10 DAA and decreased thereafter while ACC content in the peel did not widely different with fruit developmental stage (Figure 1B).

ABA, JA and sugar contents

ABA content in the peel was lower than that in the pulp. ABA content in the pulp reached maximum level of 17.2 µmol kg^{-1}FW at 20 DAA, and decreased sharply at 30 and 40 DAA (Figure 2). In contrast, ABA content in the peel did not change significantly with fruit development. On the other hand, JA content in the peel was higher than that in the pulp (Figure 3). JA content in the pulp was highest at 10 and 20 DAA and decreased sharply at 30 DAA while in the peel, JA content was higher 10 to 30 DAA and then decreased at 40 DAA. In terms of sugar changes, glucose and fructose concentrations in the pulp were initially low at 10-20 DAA and then increased sharply at 30-40 DAA (Figure 4). Sucrose content had no profound changes during fruit development. Sugar alcohols were not detected. Wichienichote *et al.* (2010) reported that *Hylocereus undatus* and *H. monacanthus* pulp contains glucose, fructose and sucrose. This data agree with our study that the pulp of dragon fruit contains glucose, fructose and sucrose whereas sugar alcohols were not detected this might because sugar alcohols such as sorbitol may converted to glucose and fructose. For instance, Gennard *et al*. (2003) reported that sorbitol were converted to glucose and fructose by sorbitol oxidase and sorbitol dehydrogenase in peach fruits respectively. Therefore, its concentration was al-ways low.

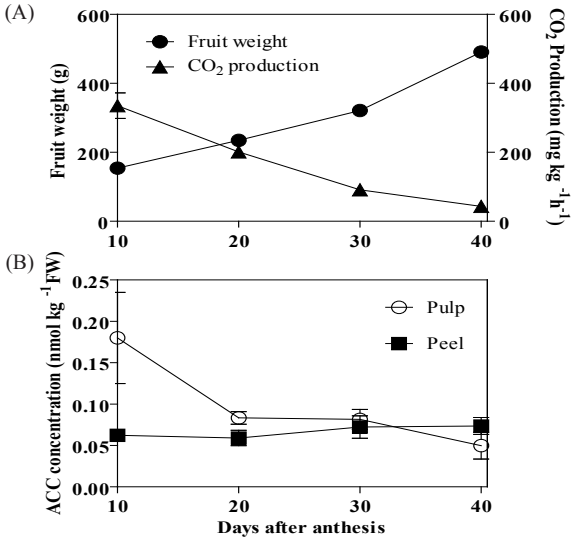

Figure 1. (A) Changes in fruit weight, CO_2 production and (B) ACC concentrations of dragon fruit during fruit development. Data are means ± SE of three replications.

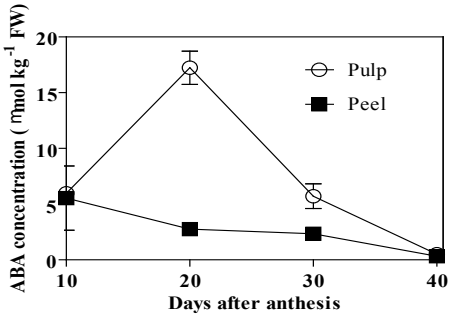

Figure 2. ABA concentrations in the pulp and peel of dragon fruit during fruit development. Data are means ± SE of three replications.

Discussion

The respiratory pattern and the changes in ACC content of dragon fruit were characte-ristic of non-climacteric type of fruit. It contrasted the climacteric pattern such that in tomato (Alexander and Grierson, 2002), banana (Dominguez-Puigjaner *et al*., 1986), avocado (Bozak *et al.*, 1990) and peach (Callahan *et al.*, 1989), in which an increasing rate of respiration and ACC accumulation during fruit development were observed. It has been reported that in climacteric fruits, especially apple, the application of exogenous ABA caused induction of ethylene biosynthesis and endogenous ABA accumulation.

Endogenous ABA concentration in the pulp of dragon fruit was initially high at 10 DAA and peaked at 20 DAA before decreasint at 30-40 DAA. This result compares well with that of Kondo and Tomiyama (1998) in non-climacteric sweet cherries in which ABA concentration increased before maturation and then decreased toward harvest. In an earlier study in mangosteen, both peel and pulp ABA was high at the early stage of fruit development and decreased

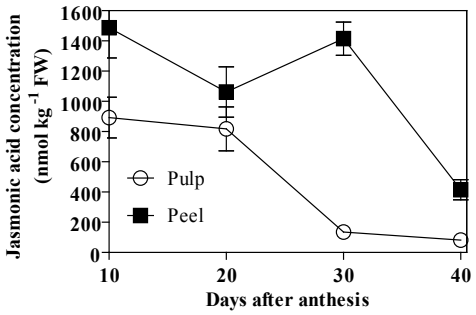

Figure 3. JA concentrations in the pulp and peel of dragon fruit during fruit development. Data are means ± SE of three replications.

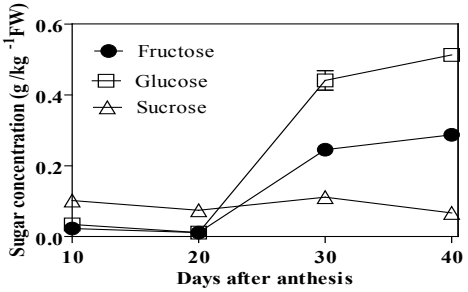

Figure 4. Sugar concentrations in the pulp of dragon fruit during fruit development. Data are means ± SE of three replications.

before peel color developed (Kondo *et al*., 2002). It has been reported that ABA is a signal molecule that promotes fruit ripening (Jia *et al.*, 2011). In addition, ABA application for inducing the development of peach fruit at 93 days after full bloom (DAFB) significantly increased sugar accumulation (Kobashi, *et al.*, 2001). In the present study, ABA concentration in the pulp increased before the rise of glucose and fructose contents. These results suggests that high ABA content at early stage of fruit development may induce the maturation of dragon fruit. The interaction between glucose signal and ABA is responsible for the induction of senescence (Wingler and Roitsch, 2008) and pigment biosynthesis (Loreti *et al*., 2008). However, our study found that ABA concentration decreased significantly at 30 DAA. This might because ABA was metabolized to β-D-glucose ester or to phaseic acid (PA) and dihydrophaseic acid (DPA). For instance, Kondo and Tomiyama (1998) reported that when ABA was applied exogenously to sweet cherries, it was metabolized to β-D-glucose ester. Therefore, the decrease in ABA content at 30 DAA may be associated with the catabolism of ABA.

The change of JA concentrations in climacteric fruit such as mangoes (Kondo *et al.*, 2004) and apples (Kondo *et al*., 2000) were high at the immature stage and the onset of ripening stage, respectively. These suggested that JA may be associated with the ripening of fruit. In sweet cherries, JA related with cell division but did not play a role on anthocyanin accumulation (Kondo *et al*., 2002). In our study, JA concentration was high in the early fruit development stage in the peel and pulp of dragon fruit and then decreased toward maturation. JA concentration in the peel was higher than that of the pulp and the decrease was delayed compared to the pulp. This may suggest that the cell division in the peel may continue longer than in the pulp because the fruit diameter increased rapidly from 10 DAA until harvest at 40 DAA. In addition, fruit peel weight was higher than that the pulp in the early fruit development stage (data not shown). A high JA content in pulp at the early stage and decline in the late stage of dragon fruit development supports the rising of fructose and glucose concentrations in pulp at 30 DAA, which indicate the fruit maturation. It is assumed that the maturation of the pulp began earlier than that of the peel.

Conclusion

The changes in respiration rate and ACC concent confirmed that dragon fruit is a non-climacteric fruit. The increase of ABA in pulp may be associated with the increase of sugar in dragon fruit and JA may be related to cell division in dragon fruit.

Acknowledgment

The authors gratefully acknowledge the financial support of the Higher Education Research Promotion and the National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand. Sincere thanks also to the Japan Student Services Organization (JASSO) for the student exchange support program.

References

Alexander, L. and Grierson, D. 2002. Ethylene biosynthesis and action in tomato : a model for climacteric fruit ripening. Journal of Experimental Botany 53: 2039-2055.

Bozak, K.R., Yu, H., Sirevag, R. and Christoffesen, R. E. 1990. Sequence analysis of ripening-related cytochrome P-450 cDANs from avocado fruit. Proceeding National Academic Science America 69: 1408-1412.

Callahan, A., Morgens, P. and Walton, E. 1989. Isolation and *in vitro* translation of RNAs from developing peach fruit. Journal of Horticultural Science 24: 356-358.

Dominguez-Puigjaner, E., Vandrell, M. and Ludevid, M.D. 1992. Differential protein accumulation in banana fruit during ripening. Journal of Plant Physiology 98: 157-162.

Gennard, M., Lescourret, F., Gomez, L. and Habib, R. 2003.

Changes in fruit sugar concentrations in response to assimilate supply metabolism and dilution: a modeling approach applied to peach fruit (*Prunus persica*). Tree Physiology 23: 373-385.

Jia, H.F., Chai, Y.M., Li, C.L., Lu, D., Lua, J.J., Qin, L. and Shen, Y.Y. 2011. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Journal of Plant Phy-siology 157: 188-199.

Kobashi, K., Sugata, S., Gemma, H. and Iwahori, S. 2001. Effect of abscisic acid (ABA) on sugar accumulation in the flesh tissue of peach fruit at the start of maturation stage. Plant Growth Regulation 35: 215-223.

Kondo, S. and Fukuda, K. 2001. Changes of jasmonate in grape berries and their possible roles in fruit development. Scientia Horticulturea 91: 275-288.

Kondo, S. and Gemma, H. 1993. Relationship between abscisic acid (ABA) content maturation of the sweet cherry. Journal of the Japan Society for Horticultural Science 62: 63-68.

Kondo, S., Inone, K. and Tamaki, M. 1999. Changes of abscisic acid and its metabolite during development of apple fruit. Journal of Horticultural Science and Biotechnology 74: 762-767.

Kondo, S. and Kawai, M. 1998. Relationship between free and conjugated ABA level in seeded and gibberellins-treated seedless, maturing 'Pione' grape berries. Journal of the American Society for Horticultural Science 123: 750-754.

Kondo, S., Motoyama, M., Michiyama, H. and Kim, M. 2002. Roles of Jasmonic acid in the development of sweet cherries as measured from fruit or disc samples. Plant Growth Regulation 37: 37-44.

Kondo, S., Ponrod, W. and Kanlayanarat, S. 2002. Abscisic acid metabolism during fruit development and maturation of mangosteens. Journal of the American Society for Horticultural Science 125 (5): 737-741.

Kondo, S., Setha, S., Rudell, S., Buchanan, D.R., and Mattheis, J.P. 2005. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. Postharvest Biology and Technology 36 :61-68.

Kondo, S. and Tomiyama, A. 1998. Changes of free and conjugated ABA in the fruit of 'Satohnishiki' sweet cherry and the ABA metabolism after application of (s)-(+)-ABA. Jour-nal of Horticultural Science and Biotechnology 73: 467-472.

Kondo, S., Tomiyama, A. and Seto, H. 2000. Changes of endogenous jasmonic acid and methyl jasmonate in apple and sweet cherries during fruit development. Journal of the American Society for Horticultural Science 123 (3): 282-287.

Kondo, S., Yazama, F., Sungcome, K., Kanlayanarat, S. and Seto, H. 2004. Change in jasmonates of mangoes during development and storage after varying harvest times. Joural of the American Society for Horticultural Science 129 (2): 152-157.

Loreti, E., Povero, G., Novi, G., Solfanelli, C., Alpi. A. and Perata, P. 2008. Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyani biosynthesis gene in Arabidopsis. New Phytologist 179: 1004-1016.

Rodrigo, M. J., Alquezar, B. and Zacarias, L. 2006. Cloning and characterization of two 9-cis-epoxycarptenoid dioxygenase gene, differentially regulated during fruit maturation and under stress conditions, from orange (*Citrus sinensis* L. Osbeak). Journal of Experimental Botany 57 : 633-643.

Setha, S. and Kondo, S. 2009. Abscisic acid level and antioxidant activity are affected by an inhibitor of cytochrome P450 in apple seedlings. Journal of Horticultural Science and Biotechnology 84: 340-344.

Saniewski, M., Nowachi, J., Lange, E. and Czapski, J. 1986. The effect of methyl jasmonate on ethylene and 1-aminocyclopropane-1-carboxylic acid production in preclimacteric and postclimacteric 'Jonathan' apple. Fruit Science Report 13: 193-2000.

Wichienchote, S., Jatupornpipat, M. and Rastall, R. A. 2010. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. Journal of Agricultural Food Chemistry 120 (3): 850-857.

Wingler, A. and Roitsch, T. 2008. Metabolic regulation of leaf senescence : interaction of sugar signaling with biotic and abiotic stress responses. Plant Biology (Suppl 1) 10: 50-62.

Zhang, M., Leng, P., Zhang, G. and Li, X. 2009. Cloning and functional analysis of 9-cis-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. Journal of Plant Physiology 16 : 1241-1252.