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SUCROSE PROMOTES STRAWBERRY FRUIT RIPENING BY STIMULATION OF ABSCISIC ACID BIOSYNTHESIS

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Abstract

The interaction of sugar with ABA plays an important role in many aspects of plant growth and development. Although glucose is reported to act as a signal molecular to promote abscisic acid (ABA) biosynthesis, whether sucrose can also serve as a signal molecule involved in ABA biosynthesis is yet unclear. Here, we showed that soluble sugars, especially sucrose, could accelerate strawberry fruit ripening both *In vivo* and *In vitro*. Based on analyses of ABA levels and transcripts of both 9-cis-epoxycarotenoid dioxygenase 1 (FaNCED1) and ABA glucosidase 1 (FaBG1) genes in developmental strawberry fruit, we found that ABA accumulation was mainly attributed to the *FaNCED1* transcripts. A fruit-tissue sucrose-incubation test indicated that sucrose could promote both *FaNCED1* and *FaBG1* transcripts evidenced by real-time PCR and SqRT-PCR. In conclusion, the main results gained from the present study suggested that sucrose might serve as a signal molecule to promote the mRNA expression levels of *FaNCED1* and plays an important role in ABA accumulation and strawberry fruit ripening.

Introduction

Fleshy fruits play an important role in our food supply, nutrition, and health. Ripening and sweetness of fleshy fruits, to a large extent, depend on sorts and contents of soluble sugars, including sucrose, fructose, and glucose (Wrolstad & Shallenberger, 1981; Akhtar *et al*., 2010; Jabbar *et al*., 2011; Demirsoy *et al*., 2012). Therefore, it is of great importance for fruit trees to develop mechanisms to regulate sugar status and carbohydrate partitioning. Notably, based on the results obtained from model plant Arabidopsis (*Arabidopsis thaliana*) in recent years, much progress has been made toward understanding the molecular mechanisms of sugar partitioning and accumulation: (1) not only finding that sugar can act as a signal molecule to play a pivotal role in plant development and stress responses (Rook *et al*., 2006; Wu *et al*., 2007); (2) but also demonstrating that the interaction of sugar with ABA is responsible for the induction of senescence (Wingler & Roitsch, 2008) starch synthesis (Baguma *et al*., 2008) , and pigment biosynthesis (Loreti *et al*., 2008); (3) in particular illustrating that glucose may modulate gene transcripts involved in both ABA biosynthesis and signaling during germination in Arabidopsis (Price *et al*., 2003; Leon & Sheen, 2003). It is well known that sucrose is the primary translocatable carbohydrate in the majority of plants, and plays an important role in sugar partitioning and accumulation (Kuhn *et al*., 1999; Coleman *et al*., 2009). However, to our knowledge, whether sucrose is involved in ABA biosynthesis is yet unclear.

Previous studies demonstrate that the primary source of ABA in plant is *de novo* synthesized by complex pathways (Nambara & Marion-Poll, 2005), in which the key regulatory step is mediated by 9-cis-epoxycarotenoid dioxygenase (NCED) that catalyzes the cleavage of 9-cis xanthophylls to xanthoxin, a precursor of ABA (Schwartz *et al*., 1997; Tan *et al*., 1997; Qin *et al*., 2002), and that the cellular ABA levels could be lowered by one conjugation pathway that forms ABA-glucosylester (ABA-GE) (Xu *et al*., 2002). Early observations suggest that ABA-GE is an inactive end product of ABA metabolism (Lehmann & Schutte, 1984; Zeevaart, 1999). However, a previous report confirms that Arabidopsis B-glucosidase1 (AtBG1) could catalyze the release of ABA-GE back into the pool of biologically active ABA to rapidly adjust ABA levels (Lee *et al*., 2006). Taken together, the main results available indicate that NCED and BG are two key enzymes for up-regulation of ABA levels in higher plants.

In the present study, the combination of a fruit-tissue sucrose-incubation test and transcription analyses of *FaNCED1* and *FaBG1* demonstrated that sucrose could promote the two gene transcripts. The present study suggested that sucrose might serve as a signal molecule to affect *FaNCED1* and *FaBG1* expression. This is the first time to address the effect of sucrose on the transcription levels of *FaNCED*1 and *FaBG*1genes in plants.

Materials and Methods

Plant materials: Octaploid strawberry (*Fragaria* x *ananassa* var. Fugilia) was planted in a greenhouse under standard culture conditions (25/15℃, 10/14 h day/night, relative humidity 70-90%). About 200 flowers on 40 strawberry plants were tagged at flowering stage. Fruits were collected on the following days (d) after anthesis: 7 d (small green), 14 d (big green), 18 d (de-greening), 21 d (white), 23 d (initial red), 25 d (partial red), and 28 d (full red). Ten fruits with uniform size were selected at each sampling time (one replication). After removing seeds, receptacle was cut into small cubes of 0.5-0.8 cm^3, then quickly froze in liquid nitrogen, and stored at –80℃ for determination of ABA, sugar, and RNA extraction.

RNA extraction, reverse transcription PCR (RT-PCR), and sequencing: Total RNA was extracted from 10 g of native or sugar treated strawberry fruits using an RNA extraction kit (SV total RNA isolation system, Promega, USA), Genomic DNA was cleared by a 15-min incubation at 37°C with RNase-Free DNase (TaKaRa, Japan.), following by RNA -Clean purification kit extraction (BioTeke Corporation, China). RNA purity and integrity were analyzed by agarose gel electrophoresis and by OD A_{260}: A_{230} and A_{260}: A_{280} ratios (a ratio of about 2.0 is

generally accepted as "pure" for RNA). To generate first-strand cDNA, total RNA (3 µg) for reverse transcription was performed using a Clontech kit (TaKaRa, Japan.) according to manufacturer's protocols. The cDNA was used as a template for amplifying *FaNCED1* with degenerate primers (forward, 5′-CT(A/T)CGCC(T/C)GC CG(G/A) TTCACCG AGAC-3′; reverse, 5′-GT(T/G) CCA(G/A)AGAT(G/C)GAAGCAGAAGCA-3′) designed by us on the basis of conserved sequences of pear, cherry, peach and grapevine *NCED1* homologues (EU912385, FJ560909, EF625684, and AY337613). PCR was performed under the following conditions: 94℃ for 3 minutes, followed by 35 cycles of 94℃ for 30s, 56℃ for 30s, and 72℃ for 1min, finally performing an extension at 72℃ for an additional 10 minutes. The PCR products were ligated into a pMD19-T vector and subsequently transformed into *Escherichia coli* DH5a. Positive colonies were selected, amplified, and then sequenced by Invitrogen China (Shanghai, China). Furthermore, with the similar approaches, the desired *FaBG1* gene were obtained using the primers (forward, 5′-GCA(C/G)TGG(A/T) TCACGT (T/G)CAATGAGCC -3′; reverse 5′-G(C/T) CCTA(T/A) CTCCA(A/G)TAGCTTTTCC-3′) designed based on conserved sequences of *BG1* homologues (XM002512051, XM002274626, AY142610, and XM002316060).

SqRT-PCR and real-time PCR assay: Total RNA was isolated from strawberry fruits as mentioned above. For SqRT-PCR analysis of *FaNCED1* and *FaBG1* gene expression, first-strand cDNA was used as a template for PCR amplification through 24 or 28 cycles for *FaNCED1* and *FaBG1* genes, respectively. These conditions were selected for comparison of relative accumulation of *FaNCED1*, *FaBG1* and *actin* mRNAs in all samples. SqRT-PCR primers were shown in Primer table below:

As for real-time RT-PCR, the reactions (20 µl) contained 10 µl SYBR Premix Ex Taq (perfect real-time containing buffer, dNTPs, $MgCl_2$ and DNA polymerase, 200 reactions, TaKaRa, Japan), 0.4 µl forward specific primer (10 µm, Sangon, China), and 0.4 µl reverse specific primer (10 µM, Sangon, China), and 2 µl cDNA template. The mixture was placed in a Bio-Rad iQ 5 Sequence Detector (USA), and DNA amplification was conducted by using the following thermocycling program: 1 cycle of enzyme activation at 95℃ for 2 minutes, 40 cycles of template denaturation at 94℃ for 20 s, primers annealing at 56℃ for 20 s, and extension at 72℃ for 30 s and 71 cycles for dissolve from 60℃ to 95℃ in the end, every cycle was 30 s for 0.5℃. All primers used for real-time RT-PCR were shown in primer table below:

Primer table.

Gene name	SqRT-PCR	Real-time PCR
FaNCED1	Fw: 5′-CCCAAACGGCACGAAAT-3′	Fw: 5′- GCCAACCCTTTACACGA-3′
	Rv: 5′-GCATCGCTCGCATTCTT-3′	Rv: 5′- TCCCGATTTCACGCTCC-3′
FaBG1	Fw: 5′- GCAACTCAAAGAGCCCAAGA-3′	Fw: 5′- GCAACTCAAAGAGCCCAAGA-3′
	Rv: 5′- TTTGAAAGGAAGGGTAATGG-3′	Rv: 5′- GAAGAATCATTACTGGCATAGAAG-3′
Actin	Fw: 5′- GTATGGTCAAGGCTGGGTT-3′	Fw: 5′- TGGGTTTGCTGGAGATGAT-3′
	Rv: 5′- CACGATTAGCCTTGGGATT-3′	Rv: 5′- CAGTTAGGAGAACTGGGTGC-3′

Note: Fw: Forward; Rv: reverse

Effect of sucrose on strawberry fruit ripening *In vivo*: A total of 200 µL sucrose, glucose and fructose (100 mM) were injected with a 0.5 mL syringe into receptacle of developing fruits (n = 20) still attached to plant alternate days for two times at 14 day after anthesis, respectively. Mannitol (100 mM) was used as a control. Seven days after injection, phenotype and ABA contents were investigated as described below.

Effect of sucrose on strawberry fruit ripening *In vitro*: Two-week –old fruits were sampled and immersed into 100mM sucrose, glucose, and fructose solutions with only their pedicels (stems attached to fruits, every kind sugar treatment used twenty fruits, n = 20, one replication) in controlled conditions (26℃, 12/12 h day/night, relative humidity 90%), respectively. Mannitol (100 mM) was used as a control. Seven days after treatment, phenotype and ABA contents were investigated as described below:

Determination of ABA content: For ABA extraction, 0.5 g receptacle was ground in a mortar and homogenized in extraction solution (80% methanol, v/v). Extracts were centrifuged at 1, 0000 g for 20 min. The supernatant liquid was eluted through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) to remove polar compounds, and then stored at –20℃ for enzyme-linked immune sorbent assay (ELISA) as described by Zhang *et al*., (2009).

Incubation of fruit disc tissue *In vitro*: Treatment on tissues of strawberry fruit with sucrose, glucose and fructose by an *In vitro* incubation was done as described by Beruter *et al*., (1995). After washing with distilled water, the freshly harvested berries were pre-cooled to 4℃. Discs of berry mesocarp, 10 mm in diameter and 1 mm in thickness, were prepared with a cork borer. The discs were immediately immersed in the equilibration buffer (Archbold, 1999) for 30 minutes with 200mM mannitol. The equilibration buffer consisted of 50 mM MES (pH 5.5), 10 mM $MgCl_2$, 10 mM EDTA, 5mM $CaCl_2$, and 5 mM Vc. The discs were divided into four sections, one section was incubated in equilibration buffer with 200 mM mannitol as control, the others were incubated in equilibration buffer with 50 mM, 150 mM and 200mM sucrose or 100mM glucose and 100mM fructose, respectively. Mannitol was used to adjust the incubation system and make it equal in osmotic potential. The four sections were placed in 250 ml Ehrlenmeyer flask and shaked at 25°C for 0.5 h, 1.5 h, 3 h, and 11 h. After washing with double distilled water, the tissues at

each time points were used immediately for assays or frozen in liquid nitrogen and kept at -80℃ until use.

Determination of soluble sugar content: Samples (25g) were taken from refrigerator (-80℃) and grinded into powder with liquid nitrogen. 0.5g powder was used and 10mL 80% ethanol was added for water bath for 3 min at 80℃, then centrifuged at 10, 000g for 10 minutes and the supernatant was collected into 100ml triangular flask. The residues were added with 10mL 80% ethanol and taken to water bath again at 80℃ for 20 minutes, then centrifuged at 10, 000g for 20 minutes. The whole process was repeated twice, and finally the supernatant was merged. The remaining residues were washed and filtered with 1mL 80% ethanol, the filtrate was moved into 10mL test tube and added with 2 drops of 5% α-naphthol and mixed, and the concentrated sulfuric acid was added slowly along its wall until there is no purple ring in the layer, which indicated that the sugar was completely extracted from the sample. The combined supernatant was evaporated in the boiling water and washed twice with 20ml distilled water, and then the volume was added up to 50ml, 2ml of which was taken for LC-18 solid-phase extraction column. The initial 1mL was abandoned and the following 1ml was collected over 0.45 μm membrane to determine soluble sugar.

The chromatographic conditions were as follows: high-performance liquid chromatography (USA), and Agilent Technologies 1200 Series, 6.5 × 300 mm Sugar-PakTM-1 column (Waters Corporation, USA), the chemical Workstation version B.02.01-SR2, ultra-pure water as mobile phase, velocity 0.4 mL/min, column temperature 60℃, refractive index detector, detector temperature: 50℃, injection volume 20μl. Standard samples used were glucose D-(+) Glucose, fructose D-(-)-Fructose, and sucrose, which were all purchased from Sigma Corporation (USA). The whole process was repeated three times. Determination results of recovery rate were 90.61% for glucose, 86.30% for fructose, 97.61% for sucrose, respectively.

Results

Sucrose shows distinct changes in developing strawberry fruit: In the current study, the developmental processes of strawberry fruit were visibly divided into seven periods: small green (SG), big green (BG), de-greening (DG), white (Wt), initial red (IR), partial red (PR), and full red (FR) at 7, 14, 18, 21, 23, 25, and 28 days after anthesis, respectively (Fig. 1). Compared with the changes of glucose or fructose in receptacle, sucrose contents was much lower at green fruits; hereafter it increased dramatically and reached its first peak value at Wt stage; with the onset of fruit coloring up, it first decline at IR stage, then rapidly increased during red-coloring stages. Possibly, because the metabolic conversion from Wt stage to IR stage needs to consume a great deal of sugar for production of pigment synthesis-related metabolites and energy, this led to sucrose content declines at IR stage after an increase in white stage. These results indicated that two rapidly rising rates of sucrose contents were coupled with fruit de-greening and red-coloring, respectively, suggesting that sucrose might play an important role in strawberry fruit ripening.

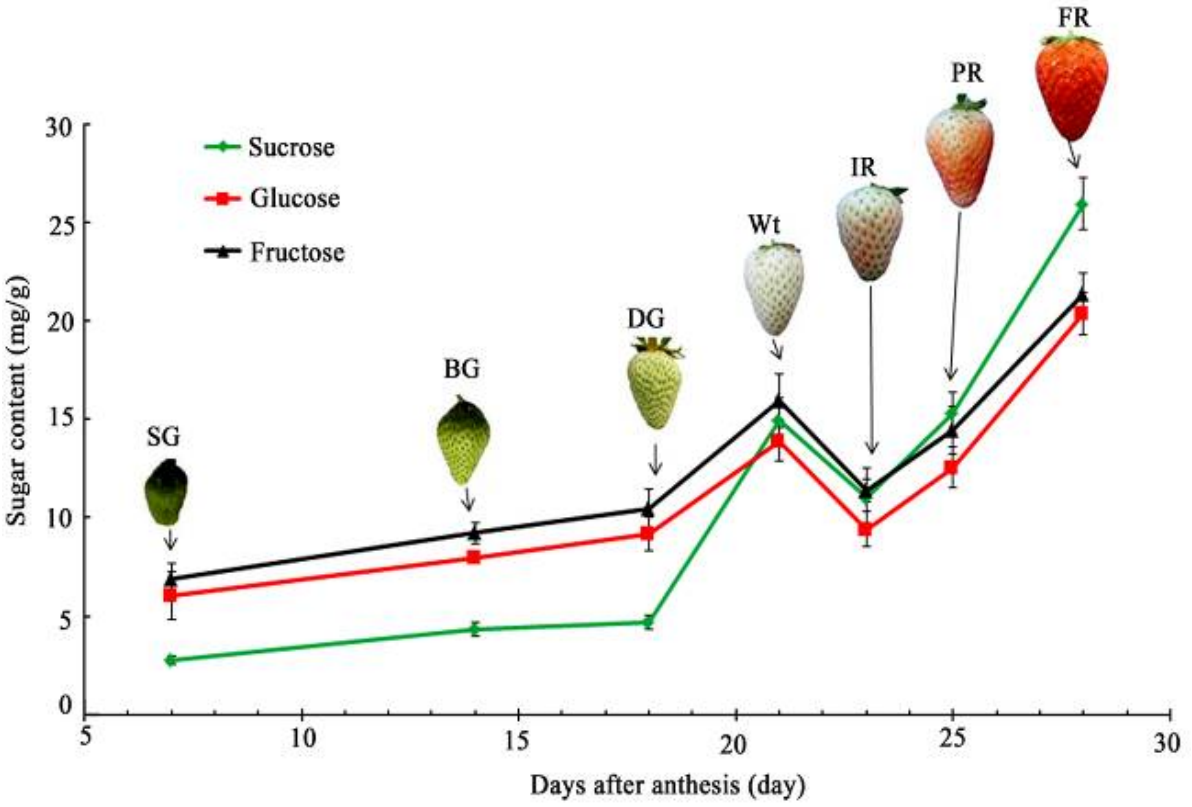

Fig. 1 Morphological and sugar changes in developmental strawberry fruit. SG, small green; BG, big green; DG, de-greening; Wt,

white; IR, initial red; PR, partial red; FR, full red. The error bars represent SE (n=3).

Sucrose promotes strawberry fruit ripening by triggering ABA accumulation *In vivo*: The investigatation of effect of sugar on strawberry fruit development showed that one week after injection, most of the sucrose-injected fruits could color up (65%); in contrast, a minority of glucose-injected fruits turned colored up (30%); few of fructose and manntiol-injected fruits could color up (less than 15%). The detection of ABA levels in receptacle of these fruits showed that ABA contents were average 62.98 ± 2.31, 64.11 ± 1.97, 72.79 ± 2.61, and 86.17 ± 2.15 ng/g in mannitol-, fructose-, glucose-, and sucrose-treated fruits, respectively (Table 1). These results revealed that sucrose could promote strawberry fruit ripening by triggering ABA levels *In vivo at maximum*.

Table 1. Effect of sugars on strawberry fruit development and ABA content both *In vivo* and *In vitro*.

Treated fruits (n=20)	Number of colored fruits		ABA contents (ng/g)	
	In vivo	*In vitro*	*In vivo*	*In vitro*
Mannitol	1a	3 a	60.12 ± 2.35 a	67.92 ± 2.37 a
Sucrose	12 b	15 b	85.79 ± 3.46 b	92.81 ± 2.54 b
Glucose	6 c	10 c	70.23 ± 3.41 c	78.54 ± 2.69 c
Fructose	2 a	4 a	63.57 ± 2.56 a	69.27 ± 2.53 a

Two-week-old strawberry fruit was used for treatment of 100 mM sucrose, glucose, and fructose both *In vivo* and *In vitro*. The phenotype was observed one week after treatment. The same lower-case letter within the same time treatment is not significantly different ($p \leq 0.05$). For analyses, SPSS 13.0 statistical packages were used

Sucrose most promotes strawberry fruit ripening by triggering ABA accumulation *In vitro*: To further confirm that sugar can promote strawberry fruit ripening, 2-week-old fruits detached from plant were immersed into100 mM sucrose, glucose, and fructose solutions with only their carpopodiums, respectively. One week after treatment, most of the sucrose-treated fruits (15/20) could color up; half of glucose-treated fruits (10/20) turn colored up; 4/20 fructose- or 3/20 manntiol-treated fruits could color up. The detection of ABA levels in receptacle of these fruits showed that ABA contents were in average of 67.92 ± 2.37, 69.27 ± 2.53, 78.54 ± 2.69, and 92.82 ± 2.54 ng/g in mannitol-, fructose-, glucose-, and sucrose-treated fruits, respectively (Table 1). These results further exhibited that sucrose could promote strawberry fruit ripening by triggering ABA levels *In vitro*.

FaNCED1 determines ABA accumulation in developmental strawberry fruit attached to plants: To explore the enzymatic regulatory mechanism of free ABA accumulation during strawberry fruit development, the mRNA expression levels of *FaNCED1* and *FaBG1* genes in receptacle were measured by real-time. The results showed that, in addition to at white stage, *FaNCED1* gene transcripts showed continual accumulation, especially had a rapid rise rate at both de-greening and red-coloring stages (Fig. 2). In contrast, the mRNA expression levels of *FaBG1* gene decreased continuously during fruit development (Fig. 2). The results showed that ABA accumulation is mainly attributed to the transcription profile of *FaNCED1*in developmental strawberry fruit.

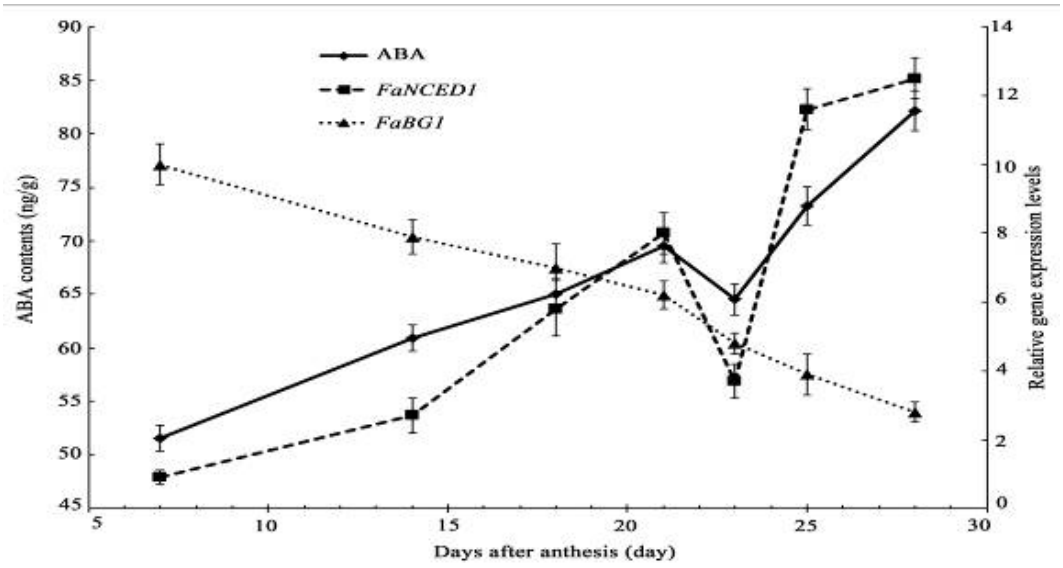

Fig. 2. Changes of ABA contents and transcripts of *FaNCED1* and *FaBG*1 genes in developmental strawberry fruit. Total RNA was extracted from 7-stage berry tissues. Total RNA (3 µg) was reversed to the first-strand cDNA for real-time PCR analysis. *Actin* was used as the internal control. The error bars represent SE (n=3).

Sucrose can promote the transcripts of both FaNCED1 and FaBG1 *In vitro*: As described above, sucrose significantly promotes strawberry fruit ripening by regulation of ABA level comparison of other sugars. Thereby, we further investigate the effect of sucrose on the mRNA expression levels of *FaNCED1* and *FaBG1* genes by a fruit tissue-incubation test *In vitro*. The results showed that sucrose significantly promoted the transcription levels of *FaNCED1* gene, compared with the control that was incubated only in equilibration buffer without sucrose, and especially, this effect was remarkably enhanced in 200mM sucrose in an 11-hr incubation as convinced by real-time PCR and SqRT-PCR (Fig. 3A). In contrast, sucrose significantly inhibited the mRNA expression levels of *FaBG1* gene at early incubation stage (0.5 h) compared with the control (Fig. 3B, black column). Interestingly, this inhibited effect was released after 1.5 h incubation stage (Fig. 3B, red, yellow, and blue column), and especially, this effect was remarkably enhanced in 200mM sucrose after 11-hr incubation. These results demonstrated that sucrose significantly could promote the transcripts of both *FaNCED1* and *FaBG1* genes.

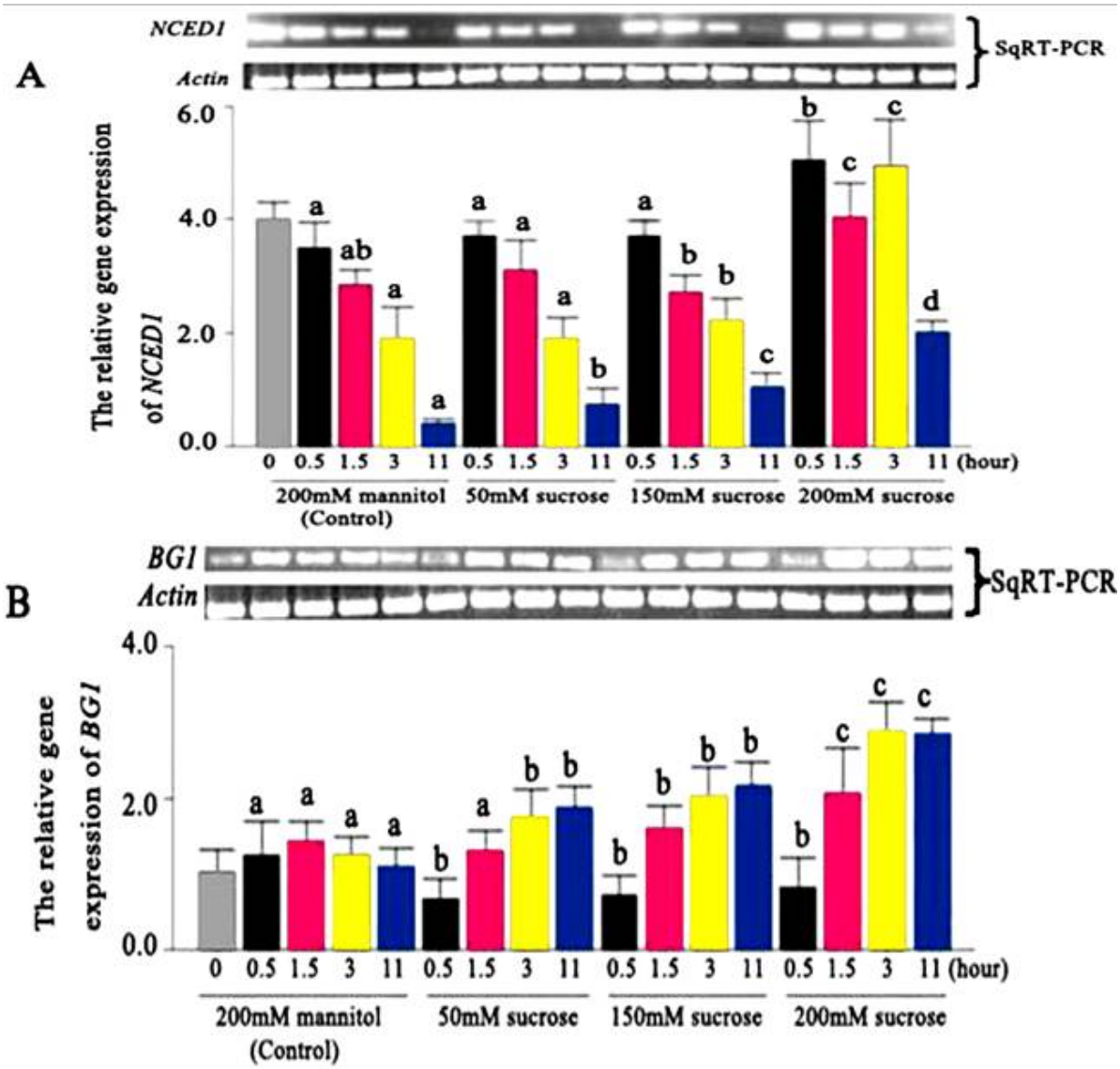

Fig. 3. Determination of effect of sucrose on *FaNCED1* and *FaBG1* transcripts using a fruit tissue-incubation test. Strawberry tissues were pre-incubated in equilibration buffer including 200 mM mannitol for 0.5 h, which then were divided into four parts (control, 0 h). One part is incubated continually in equilibration buffer, the other three parts are incubated in medium containing different concentrations of sucrose from 50, 150, to 200 mM respectively. After incubation of 0.5 h, 1.5 h, 3 h, and 11 h, total RNA was extracted from the treated tissues. rRNA indicated loading control of the RNA samples stained with ethidium bromide (A). Total RNA (3 µg) was reversed to the first-strand cDNA for SqRT-PCR and real-time PCR analysis. The abundance of *FaNCED1* and *FaBG1* transcripts was evaluated using 24 (A) and 28 (B) cycles of RT-PCR with *FaNCED1* and *FaBG1*-specific primers, respectively. *Actin* was used as the internal control. The error bars represent SE (n=3). Columns with the same lower-case letter within the same time treatment are not significantly different ($p \leq 0.05$).

Discussion

Strawberry is referred to, in the horticulture, enlarged receptacle with achenes, which developed and ripen rapidly (about 30 days from anthesis to ripeness in our growth conditions). Based on a previous report on a six-stage division of strawberry fruit development: small green (SG), medium green (MG), big green (BG), white (Wh), turning (Tu), and red (Fait *et al*., 2008), we visually divided these processes into seven different stages: small green (SG), big green (BG), de-greening (DG), white (Wh), initial red (IR), partial red (PR, namely Tu), and full red (FR). The combination of fruit developmental processes and sugar content analyses suggests that sucrose might play an important role in the regulation of strawberry fruit ripening rather than glucose or fructose. Interestingly, a series of exogenous sugar-treated tests both *In vivo* and *In vitro* demonstrated that among three soluble sugars, sucrose could most promote the fruit ripening by triggering ABA levels. Notably, previous reports also indicate that strawberries are defined as non-climacteric because they do not exhibit a peak in respiration and ethylene production during ripening, and the application of ethylene to green strawberries does not affect the rate of ripening (Knee *et al*., 1977; Given *et al*., 1988; Abeles & Takeda, 1990). In contrast, exogenous ABA markedly promotes the fruit maturation (Kano & Asahira, 1981; Manning, 1994; Perkins-Veazie, 1995; Jiang & Joyce, 2003). The significant correlation coefficients of ABA-sucrose, ABA-*FaNCED1* and sucrose- *FaNCED1* (0.92, 0.91, 0.86, respectively, data not listed) suggest that sucrose might serve as a signal molecule to trigger ABA biosynthesis.

In past decades, the interaction of ABA with sugar is extensively studied in model plant Arabidopsis and finding that sugars can induce the accumulation of anthocyanins in a ABA-synergistic manner (Mita *et al*., 1997; Jeannette *et al*., 2000; Loreti *et al*., 2008; Solfanelli *et al*., 2006) and that sugar related-mutants are involved in either ABA biosynthesis or ABA signaling (Gibson, 2005; Leon & Sheen J, 2003). For example, Cheng *et al*., (2002) find that the transcription levels of several ABA biosynthesis genes are promoted by glucose only at low concentrations (2%), and this effect is not further enhanced with higher glucose levels. However, to our knowledge, whether sucrose also may serve as a signal molecular involved in ABA biosynthesis was largely unclear in the past. Interestingly, in the current study, a fruit-tissue sucrose-incubation test also validated that sucrose significantly promoted the mRNA expression levels of *FaNCED1* and *FaBG1* gene, and this effect was further remarkably enhanced with both high sucrose concentration (200 mM, about 7%) and long-time incubation (above 1.5 h). Given that ABA accumulation in developmental strawberry fruit is mainly attributed to *FaNCED1*, although ABA can promote *FaBG1* transcripts, we consider that the role of BG1 is main responsible for strawberry fruit early development rather than ripening by fine-tuning ABA levels; in contrast, the role of FaNCED1 is on the contrary in this situation.

Conclusion

The main conclusion gained from the present study is that a rapid rising rate of sucrose is coupled with de-greening and red-coloring in developmental strawberry fruit; exogenous sucrose can most accelerate ripening by promoting ABA accumulation; ABA accumulation is mainly attributed to *FaNCED1*transcripts. Reasonably, the plant recruits sucrose *per se* to act as a signal molecule to modulate ABA biosynthesis by FaNCED1, in turn, this hormone evokes a rapid response to sugar accumulation and then regulates fruit ripening. These results also demonstrate that the interaction between sugar and ABA plays an important role in strawberry fruit ripening.

Acknowledgements

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References

Abeles, F.B. and F. Takeda. 1990. Cellulase activity and ethylene in ripening strawberry and apple fruits. *Sci. Hort.*, 42: 269-275.

Akhtar, I., N.A. Abbasi and A. Hussain. 2010. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Pak. J. Bot*., 42(1): 181-188.

Archbold, D.D. 1999. Carbohydrate availability modifies sorbitol dehydrogenase activity of apple fruit. *Physiol. Planta*., 105: 391-395.

Baguma, Y., C. Sun, M. Borén, H. Olsson, S. Rosenqvist, J. Mutisya, P.R. Rubaihayo and C. Jansson. 2008. Sugar-mediated semidian oscillation of gene expression in the cassava storage root regulates starch synthesis. *Plant Signal Behav*., 3: 439-445.

Beruter, J. and F.M.E Studer. 1995. Comparison of sorbitol transport in excised tissue discs and cortex tissue of intact apple fruit. *J. Plant Physiol*., 146: 95-102.

Cheng, W.H., A. Endo, L. Zhou, J. Penney, H.C. Chen, A. Arroyo, P. Leon, E. Nambara, T. Asami, M. Seo, T. Koshiba and J. Sheen. 2002. A unique short-chain dehydrogenase/reductase in Arabidopsis abscisic acid biosynthesis and glucose signaling. *Plant Cell*, 14: 2723-2743.

Coleman, H.D., J. Yan and S.D. Mansfield. 2009. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proc. Natl. Acad. Sci*., 106: 13118-13123.

Demirsoy, L., H. Demirsoy and G. Balci. 2012. Different growing conditions affect nutrient content fruit yield and growth in strawberry. *Pak. J. Bot.*, 44(1): 125-129.

Fait, A., K. Hanhineva, R. Beleggia, N. Dai, I. Rogachev, V.J. Nikiforova, A.R. Fernie and A. Aharoni. 2008. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiol*., 148: 730-750.

Gibson, S.I. 2005. Control of plant development and gene expression by sugar signaling. *Curr. Opin. Plant Biol*., 8: 93-102.

Given, N.K., M.A. Venis, and D. Grierson. 1988. Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta*, 174: 402-406.

Jabbar, A., A.U. Malik, Islam-Ud-Din, R. Anwar, M. Ayub, I.A. Rajwana, M. Amin and A.S. Khan. 2011. Effect of combined application of fungicides and hot water quarantine treatment on postharvest diseases and quality of mango fruit. *Pak. J. Bot.*, 43(1): 65-73.

Jeannette, E., A. Reyss, N. Grégory, P. Gantet and J.L. Prioul. 2000. Carbohydrate metabolism in a heat-girdled maize source leaf. *Plant Cell and Environ*., 23: 61-69.

Jiang, Y. and D.C. Joyce. 2003. ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. *Plant Growth Regul*., 39: 171-174.

Kano, Y. and T. Asahira. 1981. Roles of cytokinin and abscisic acid in the maturing of strawberry fruits. *J. Japan Soc. Hort. Sci*., 50: 31-36.

Knee, M., J.A. Sargent and D.J.Osborne. 1977. Cell wall metabolism in developing strawberry fruits. *J. Exp. Bot*., 28: 377-396.

Kuhn, C., V.R. Franceschi, A. Schulz, R. Lemoine and W.B. Frommer. 1999. Update on sucrose transport in higher plants. *J. Exp. Bot.*, 50: 935-953.

Lee, K.H., H.L. Piao, H.Y. Kim, S.M. Choi, F. Jiang, W. Hartung, I. Hwang, J.M. Kwak, I.J. Lee and I. Hwang. 2006. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell*., 126: 1109-1120.

Lehmann, H. and H.R. Schutte, 1984. Abscisic-acid metabolism in intact wheat seedlings under normal and stress conditions. *J. Plant Physiol*., 117: 201-209.

Leon, P. and J. Sheen. 2003. Sugar and hormone connections. *Trends Plant Sci*., 8: 110-116.

Loreti, E., G. Povero, G.. Novi, C. Solfanelli, A. Alpi and P. Perata. 2008. Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. *New. Phytol*., 179: 1004-1016.

Manning, K. 1994. Changes in gene expression during strawberry fruit ripening and their regulation by auxin. *Planta*, 94: 62-68.

Mita, S., N., Murano, M. Akaike and K. Nakamura. 1997. Mutants of Arabidopsis thaliana with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that are inducible by sugars. *Plant J*., 11: 841- 851.

Nambara, E. and A. Marion-Poll. 2005. Abscisic acid biosynthesis and catabolism. *Ann. Rev. of Plant Physiol*., 56: 165-185.

Perkins-Veazie, P. 1995. Growth and ripening of strawberry fruit. *Hort Rev*., 17: 267-297.

Price, J., T.C. Li, S.G. Kang, J.K. Na and J.C. Jang. 2003. Mechanisms of glucose signaling during germination of Arabidopsis. *Plant Physiol*., 132: 1424-1438.

Qin, X.Q. and J.A.D. Zeevaart. 2002. Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in Nicotiana plumbaginifolia increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiol.*, 128: 544-551.

Rook, F., S.A. Hadingham, Y. Li and M.W. Bevan. 2006. Sugar and ABA response pathways and the control of gene expression. *Plant Cell Environ*., 29: 426-434.

Schwartz, S.H., B.C. Tan, D.A. Gage, J.A.D. Zeevaart and D.R. Mccarty. 1997. Specific oxidative cleavage of carotenoids by VP14 of maize. *Science*, 276: 1872-1874.

Solfanelli, C., A. Poggi, E. Loreti, A. Alpi and P. Perata. 2006. Sucrosespecific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant Physiol*., 140: 637-646.

Tan, B.C., Schwartz, S.H. J.A.D. Zeevaart and D.R. Mccarty. 1997. Genetic control of abscisic acid biosynthesis in maize. *Proc. Natl. Acad. Sci.*, 94: 12235-12240.

Wingler, A. and T. Roitsch. 2008. Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. *Plant Biol. (Stuttg)*., 1: 50-62.

Wrolstad, R.E. and R.S. Shallenberger. 1981. Free sugars and sorbitol in fruits. A compilation from the literature. *J Assoc Off Anal Chem.*, 64: 91-103.

Wu, Z., X. Mo and P. Wu. 2007. AtCYT-INV1 in Arabidopsis Sugar Signaling. *Plant Signal Behav*., 2: 496-497.

Xu, Z.J., M. Nakajima, Y. Suzuk and I. Yamaguchi. 2002. Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. *Plant Physiol*., 129: 1285-1295.

Zeevaart, J.A.D. 1999. Abscisic acid metabolism and its regulation. In: *Biochemistry and Molecular Biology of Plant Hormones*. (Eds.): P.J.J. Hooykaas, M.A. Hall and K.R. Libbenga. Amsterdam: Elsevier, pp. 189-207.

Zhang, M., B. Yuan and P. Leng. 2009. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. *J. Exp. Bot.*, 60: 579-1588.

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