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Comparative Transcript Levels of Sugar Metabolism Genes Between the Canary Melon and a Vietnamese Non-Sweet Melon Cultivar

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ABSTRACT

Two different melon cultivars, Canary and Vietnamese non-sweet melons, are used to compare the fruit's sweetness levels. The results indicate that the Canary melon is much sweeter than the non-sweet melon. The transcript levels of the sugar metabolism genes, including *Cucumis melo ACID INVERTASE 2* (*CmAIN2*) and *SUCROSE SYNTHASE 1* (*CmSUS1*), are examined in two fruit tissues. PCR using cDNA and the electrophoresis assays indicate that the *CmAIN2* and *CmSUS1* primer sets are specific, and only one band of PCR product is obtained from all tested samples. The quantitative reverse transcription PCR (RT-qPCR) assay is applied to compare the transcript levels of the *CmAIN2* and *CmSUS1* genes in fruit tissues of the Canary and the Vietnamese non-sweet melons. Consistent with the sweetness levels, the *CmAIN2* and *CmSUS1* transcript levels are higher in the Canary melon than those in the non-sweet melon. These results imply that the local sugar metabolism in the fruits may also play an essential role in determining fruit sweetness. In addition, practically, the transcript levels of the *CmAIN2* and *CmSUS1* genes can be accessed and used to predict the sweetness of melon fruits early.

INTRODUCTION

Canary melon has been widely consumed as a dessert fruit due to its fragrance, sweetness, and flavorful taste. However, the cultivation of this cultivar has been challenged in Vietnam because of its weak growth performance and high susceptibility to local pathogens (Nguyen et al., 2023a).

Fruit ripening and softening are vital traits affecting food supply, fruit nutritional value and human health. Worldwide, melon fruits have been consumed by humans as a dessert fruit. Besides the flavorful taste, melon fruits are known to contain many beneficial natural compounds for humans, such as vitamins (A and C) and other bioactive compounds (Dahmani-Mardas et al., 2010; Monforte et al., 2014; Gómez-García et al., 2020; Amzeri et al., 2022). Sweetness (sucrose accumulation) is a key characteristic determining melon fruit quality and consumption (Dai et al., 2011; Schemberger et al., 2020).

It has been suggested that a reduction of glucose and fructose accompanies the sucrose accumulation, and it is plausible to propose that sucrose can be biosynthesized from glucose and fructose in some members of the *Cucurbitaceae* family (Chrost & Schmitz, 1996; Dai et al., 2011). Previously, different genes involved in sucrose metabolism were elucidated (Dai et al., 2011; Schemberger et al., 2020). For instance, *SUCROSE SYNTHASEs* (*SUSs*), *ACID INVERTASEs* (*AINs*), *INVERTASE INHIBITORs* (*INHs*), *UDPGLC EPIMERASEs* (*UGEs*), *SUCROSE-P SYNTHASE 2* (*SPS2*) encode for different enzymes involved in the sucrose metabolism in the melon fruits (Dai et al., 2011; Schemberger et al., 2020).

In prior works, the sucrose transport from the source (leaves) to the sink (fruits) has been suggested to play an important role more than *de novo* synthesis in the sink organs (fruits) (Hackel et al., 2006; Jia et al., 2013; reviewed in Durán-Soria et al., 2020). On the other hand, studies on sugar

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metabolism in melon fruits are still limited (Dai et al., 2011; Schemberger et al., 2020). In this study, two different melon cultivars, including a sweet melon (Canary) and a local non-sweet melon, were used to examine the transcript levels of a gene (namely *CmAIN2* and *CmSUS1*) involved in sucrose metabolism. The fruit tissues (exocarp and mesocarp) were carefully isolated from the fruits with different developmental stages (7, 28, and 35 days after pollination) and used to compare the *CmAIN2* and *CmSUS1* transcript levels.

The research employed two different melon cultivars, namely Canary and Vietnamese non-sweet melons. The study compares the fruit sweetness levels by examining two fruit tissues.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The seeds of the Canary and the local non-sweet melons were sown and grown in the experimental garden, Laboratory of Plant Cell Biotechnology, Ho Chi Minh City Open University, Binh Duong Campus, Vietnam, in 2022. Subsequently, the fruits were collected and used for further studies.

Sensory Analysis

The fruits were collected on the indicated date and used for sensory analysis to evaluate the sweetness level. A group of 5 people was employed to assess the melons' sweetness. The test was performed blindly, and the assessment scoring system is shown in Table 1.

Table 1. Sensory assessment scoring for fruit sweetness

Score	Description
1	Not sweet
2	Very slightly sweet
3	Slightly sweet
4	Sweet
5	Very sweet

RNA Extraction, First-Strand cDNA Synthesis, and PCR

After pollination, the melon fruits at different developmental stages (7, 28, and 35 days) were collected for RNA extraction from the exocarp and mesocarp tissues. The total reagent isolated the TRIzol RNA (TRIzol™ Reagent, Invitrogen™, Thermo Fisher Scientific, Massachusetts, USA) (Simms et al., 1993; Vennapusa et al., 2020). Next, the obtained RNA samples proceeded to the first-strand cDNA synthesis using the SensiFAST cDNA Synthesis Kit (Cat. No. BIO-65054, Meridian Bioscience, Ohio, USA) as instructed by the manufacturer. The cDNA samples were used for PCR reactions with indicated primers (Table 2).

Table 2. List of primers used in this study

Gene name	Sequence (5' – 3')
CmCLO	F – GGTTCAAGTCCCTCTATCCC
	R – ATTTGAACTGGTGACACGAG
CmAIN2	F – CTCGATCTTGCAAAAAGCAGC
	R – TTCCCCAAACGCCCCTAAAG
CmSUS1	F – AACCGTGTTCATAGCCTCCG
	R – TCAGGACTTCCCCAAATGCC

Real-Time PCR (qPCR)

In addition to PCR, the above cDNA samples were employed for qPCR as described previously with some modifications (Nguyen & Cheong, 2018). The detailed PCR cycling program includes: (1) 95°C/5 minutes (1 cycle); (2) 95°C/30 seconds and 60°C/30 seconds (40 cycles); (3) Melting curve analysis (Nguyen et al., 2023b). The primers used for qPCR are mentioned in Table 2. The Caleosins (CLO) gene was included as an internal control. The 2-ΔΔCT method was applied to calculate the relative transcript levels (Livak & Schmittgen, 2001). The assay was biologically repeated three times, with three replicates each time.

RESULTS AND DISCUSSION

Comparison of the Sweetness Levels Between the Canary and Non-Sweet Melons

In this study, sensory analysis is employed to compare the sweetness levels between the Canary and non-sweet melons (Fig. 1a.) As shown in Fig. 1b, the sweetness of the Canary melon gradually increases during the growth of the fruits. On the other hand, the sweetness of the non-sweet melon is almost unchanged and lower compared to the Canary melon (Fig. 1b). The obtained results further confirm that the Canary melon is much sweeter than the non-sweet one. In a previous study, the hybrid lines were generated from the crossing between these Canary and non-sweet melons, and the soluble sugars analysis indicated that the hybrid lines' sweetness levels were changed based on the mother line (Nguyen et al., 2023a). In addition,

this study also found that the Canary melon fruits accumulated higher sucrose levels than those of the non-sweet melon ones (Nguyen et al., 2023a). This work further confirms that the Canary melon is sweeter than the non-sweet melon, and this phenotypic observation is an important background for the following molecular biological analysis.

Specificity of *CmAIN2*, *CmSUS1*, and *CmCLO* Primer Sets in Exocarp and Mesocarp Tissues of the Canary and Non-Sweet Melons

To examine the specificity of the primer sets for two genes, *CmAIN2* and *CmSUS1*, the PCR assay was carried out with cDNA samples obtained from exocarp and mesocarp tissues of the Canary and non-sweet melon fruits. The PCR products (namely, *CmAIN2* and *CmSUS1* genes) indicated only one band for each sample (Fig. 2a-b). In the subsequent experiment (RT-qPCR), the *CmCLO* gene is used as an internal control. Thus, a PCR assay is also carried out to test the specificity of the *CmCLO* primers. As shown in Fig. 2c, the PCR products (for the *CmCLO* gene) also exhibited one band. Some studies have been carried out on the melon species. However, the amount of work is still limited.

On the other hand, the variation between cultivars or varieties can differ in genomic sequences. Therefore, before processing the qPCR, it is crucial to test the specificity of the designed primers (generally based on the DNA sequences obtained from a specific melon cultivar/variety). This study does the PCR and checks the results on the gel electrophoresis to solidly confirm that all primers are working well and are highly specific. As expected, all primers are specifically associated with the template DNAs from the Canary and the non-sweet melons (Fig. 2) and are suitable for further experiments (RT-qPCR).

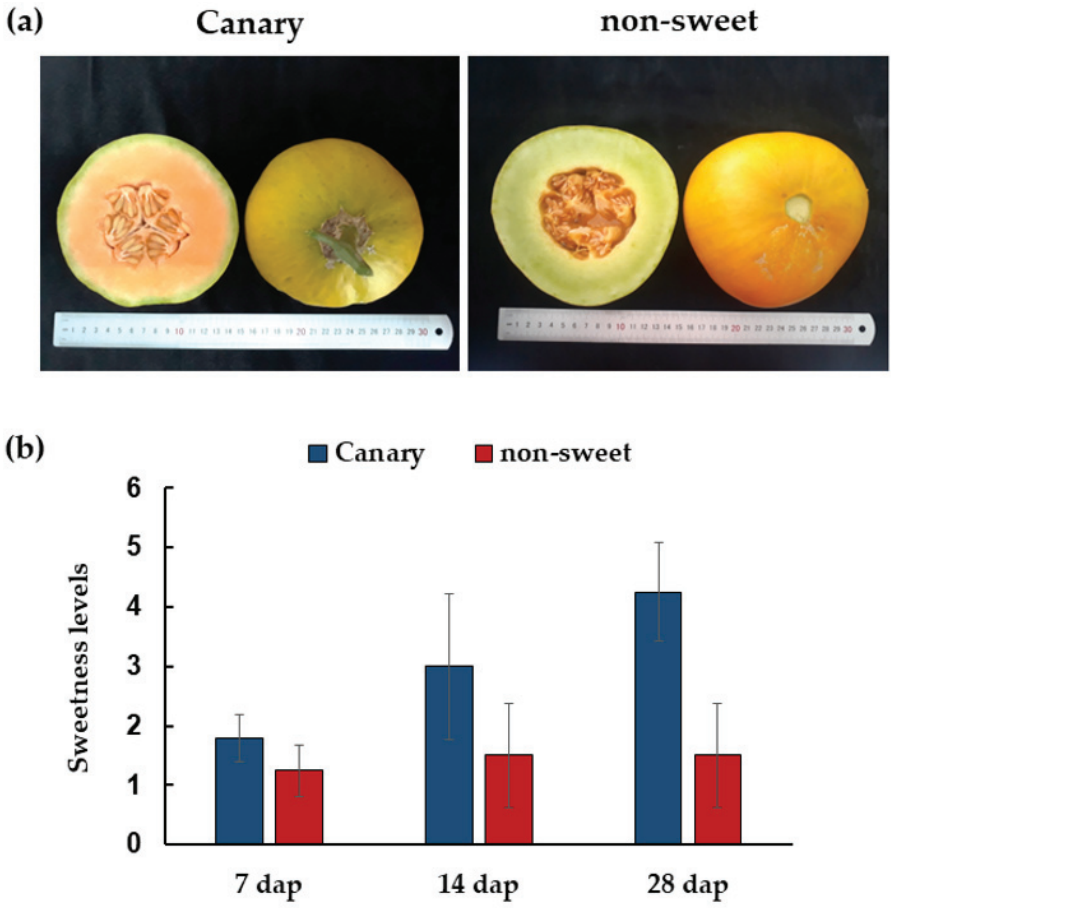
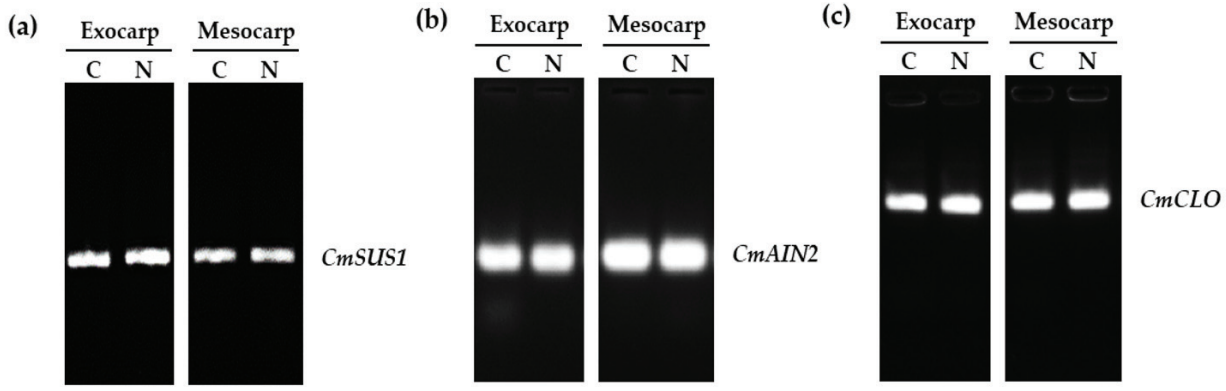


Fig. 1. Comparison of the sweetness between the Canary and non-sweet melons: (A) The Canary and non-sweet melons fruits (28 dap [day after pollination]), (B) Sweetness levels of the Canary and non-sweet melons during fruit development.

Comparative Transcript Levels of *CmAIN2* and *CmSUS1* Between the Canary and the Non-Sweet Melon Cultivars

Two different melon cultivars were selected to study the correlation between the sweetness and the transcript levels of genes involved in the sugar metabolism in the melon fruits, including the Canary melon (sweet melon) and the Vietnamese non-sweet melon. Next, the fruits with different developmental stages [7, 28, and 35 days after pollination (dap)] were also harvested for RNA extraction. As shown in Fig. 3, *CmAIN2* and *CmSUS1* transcript levels are found to be higher in the exocarp tissue of the Canary melon fruit than those in the non-sweet melon at the young fruit stage (7 dap). However, during the older fruit stages, such as 28 and 35 dap, the *CmAIN2* and *CmSUS1* transcript levels are almost similar between the two tested cultivars (Fig. 3). Besides, it can be seen the decline of the *CmAIN2* and *CmSUS1* transcript levels in the exocarp tissue of the Canary melon fruit when the fruits are getting older (highest in 7 dap fruit while very low in 28 and 35 dap fruits) (Fig. 3). Previous studies reviewed that the transition of the melon fruit from the early stage to the sucrose accumulation stage is associated with a developmental loss of the activity of the soluble enzyme, acid invertase (Burger et al., 2009; Dai et al., 2011). In addition to the loss of enzyme acid invertase activity, previous work also found that the mRNA levels of *CmAIN2* and *CmSUS1* were gradually decreased from the young stage to the sucrose accumulation stage fruits (Dai et al., 2011). This is consistent with the results obtained in this study (Fig. 3).



Remarks: C: Canary melon; N: non-sweet melon

Fig. 2. The specificity of the primer sets used in this study. PCR and electrophoresis assays indicated the primers for *CmAIN2* (A), *CmSUS1* (B), and *CmCLO* (C) genes were highly specific for the tested samples

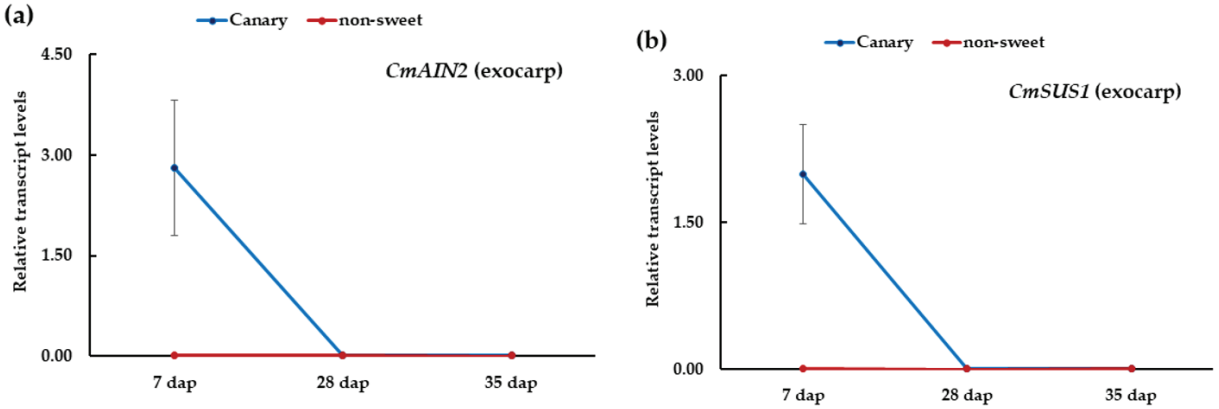


Fig. 3. *CmAIN2* and *CmSUS1* transcript levels in the exocarp tissue samples of the Canary and non-sweet melons. Data were obtained from three independent experiments (3 replicates/each).

However, this phenomenon was scarcely observed in the non-sweet melon, as the *CmAIN2* and *CmSUS1* genes exhibited relatively low expression in the exocarp of non-sweet melon fruit, with minimal variation across different fruit developmental stages. These results suggest that the expression of *CmAIN2* and *CmSUS1*, along with the corresponding enzymes, may play a crucial role in sucrose accumulation in melon fruits, ultimately influencing fruit sweetness.

In tomato fruit, variations in transcript levels were observed among *SUCROSE TRANSPORTER (SUT)* genes across different fruit tissues, including the exocarp, mesocarp, and endocarp (Sun et al., 2022). Beyond the examination of exocarp tissue (Fig. 3), this study further investigated the transcript levels of *CmAIN2* and *CmSUS1* in the mesocarp tissue of the fruits (Fig. 4). Interestingly, the expression levels of these genes are relatively low in the mesocarp tissue of both cultivars (Fig. 4). Despite the subdued expression in the mesocarp tissue, the trends of *CmAIN2* and *CmSUS1* transcript levels were similar between the two cultivars, gradually decreasing from the young to old stages of the fruits (Fig. 4).

It has been suggested that the sucrose transport from the source (leaves) to the sink (fruits) is more important than *de novo* synthesis in the sink organs (fruits) (Hackel et al., 2006; Jia et al., 2013; reviewed in Durán-Soria et al., 2020). Besides a few reported studies, research on sugar metabolism in melon fruits is still limited (Dai et al., 2011; Schemberger et al., 2020). The present work shows that the expression of the genes involved in sucrose metabolism differs between two melon cultivars (sweet melon – Canary and the non-sweet one). The results also imply that the local sucrose metabolism in fruit may also play an important role in determining fruit sweetness. In addition, it is possible to test the expression of sucrose metabolism genes (e.g., *CmAIN2* and *CmSUS1*) in the young fruit stage to predict the sweetness levels of the fruits.

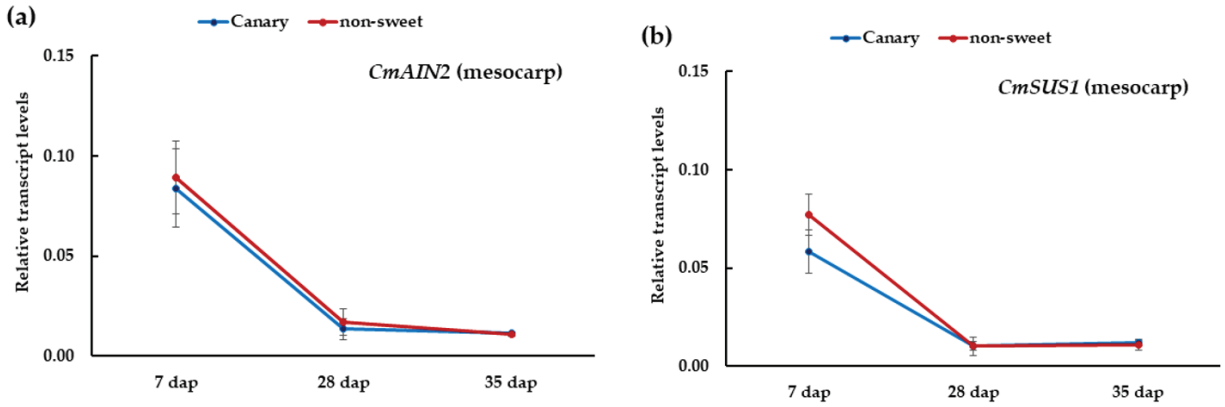


Fig. 4. *CmAIN2* and *CmSUS1* transcript levels in the mesocarp tissue samples of the Canary and non-sweet melons. Data were obtained from three independent experiments (3 replicates/each).

CONCLUSION AND SUGGESTION

In the present work, the results suggest that the fruit-local sugar metabolism may also contribute to determining fruit sweetness. Besides, the RT-qPCR method can be applied to check the transcript levels of *CmAIN2* and *CmSUS1* in the young fruit tissues to predict the sweetness of melon fruits practically.

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