Establishment of protocol to investigate the expression of *Sucrose Phosphate Synthase 2 (SPS2)* in Vietnamese golden melon (*cucumis melo* L.)

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ABSTRACT

The accumulation of soluble sugars, especially sucrose, in mature Vietnamese golden melon, has been reported to be the determining factor of the sweetness of fruits. The gene of *SPS2* has been shown to be mainly involved in the metabolic signal pathway as those for the synthesis of sucrose. The purpose of the current study is to establish a procedure to investigate the expression of the gene of *SPS2* in *Cucumis melo* L. The materials of melon's exocarp and mesocarp tissues were applied to isolate the total of RNAs. The procedure of Real-time PCR within the primers of SPS-F and SPS-R was successfully established as seen through the results. As a result, the cycle threshold (Ct) of 27.19, and 28.12, respectively, were observed in the sample of exocarp and mesocarp tissues. For authenticity, the products were sequenced, as determined as the gene of *SPS* by BLAST (NCBI). In conclusion, the procedure of investigation of *SPSs* gene expression was successfully established in *Cucumis melo* L.

1. Introduction

Golden melon, scientifically known as *Cucumis melo* L., belongs to the family of *Cucurbitaceae*, and is a worldwide fruit-bearing vegetable species with the characteristic of short growth as well as high productivity. The fruit of melon has been reported as naturally low in fat and sodium and has no cholesterol. It offers multiple benefits to human health due to the presence of many essential nutrients such as phosphorus (21mg/100g), calcium (11mg/100g), iron (0.6mg/100g), and carbohydrates (4.2g/100g) (Gene, 1997; Manchali, Murthy, Vishnuvardara, & Patil, 2021). Additionally, due to its high water content (93.3g/100g), it is a healthy option for human health (Rodriguez, Shaw, & Cantliffe, 2007). The plants of melon grow and mature quite slowly, and they are sensitive to pests and illnesses. Therefore, they are vulnerable to pest assaults, especially during the rainy season. However, golden melon fruits are of excellent quality, with sweet and crisp flesh and distinctive aromas. In order to ensure the plants' healthy growth and development, farmers must use excellent cultivation practices.

According to a study by Leida et al. (2015), during the ripening process, fruits of golden melon undergo various biochemical and physiological changes, especially the accumulation of sucrose, which has been reported to be the main determining factor that affects the sweetness of fruit (Leida et al., 2015). Sugar accumulation, especially sucrose, is an important process that determines the fruit's sweetness and is controlled by two processes: sugar translocation and metabolic exchanges during fruit development. The process of sucrose accumulation involves 53 genes, related to fruit ripening, the accumulation of sugar that contributes to the sweet taste of ripe fruits (Chen, Zhang, Li, Qin, & Tian, 2021; Durán-Soria, Pott, Osorio, & Vallarino, 2020).

According to the synthesis of sucrose, sucrose phosphate synthase (SPS, EC 2.4.1.14) has been reported to be the key enzyme involved in sucrose production and accumulation in fruit, including melon (Anur, Mufithah, Sawitri, Sakakibara, & Sugiharto, 2020; Hubbard, Huber, & Pharr, 1989; Liao et al., 2022). As function, SPS catalyzes the conversion of uridine diphosphate glucose (UDPG) and fructose-6-phosphoric acid (F6P) to sucrose-6-phosphoric acid (S6P), which is then irreversibly converted to sucrose by sucrose phosphatase (Liao et al., 2022; Lunn, 2003). Concerning the melon, the enzyme of SPS has increased strongly at the end of the growing stage, combining with the activities of invertase, resulting accumulation of sucrose (Hubbard et al., 1989). In current study, aims to establish the protocol to investigate the expression of *SPS* in *Cucumis melo* L. for further studies related to the enzymes of accumulation of sucrose.

2. Materials and methods

Sample collection

The fruits at the developmental stage of the day 28 days after pollination were harvested and used for isolating the exocarp and mesocarp tissues. The post-collected tissues were stored at a temperature of -80^{o}C for further studies.

RNA isolation, real-time PCR assay, sequencing

A total of RNA was isolated from the exocarp and mesocarp tissues by using the method of TRIzol (pH = 4) (Simms, Cizdziel, & Chomczynski, 1993). The cDNA was converted by using SensiFAST cDNA Synthesis Kit (Cat. No. BIO-65054) as instructed by the manufacturer. The reverse-transcription assay was conducted following thermal cycles of (1) 25^{o}C for 10 minutes; (2) 42^{o}C for 15 minutes; and (3) 85^{o}C for 05 minutes. The purity and concentration of cDNA were evaluated by optical density.

The obtained cDNA samples were also used for qPCR and the detailed procedure of the qPCR assay was described previously (Nguyen & Cheong, 2018). The detailed sequences of the primers used in this study are listed in Table 1. The thermal cycle of Real-time PCR was conducted following procedure: (1) 95^{o}C for 05 minutes; 40 cycles for (2) 95^{o}C for 30 seconds; (2) 60^{o}C for 30 seconds. Finally, 5μL aliquots of amplification product were electrophoresed on a 2.0% agarose gel and visualized in a UV transilluminator. The amplified product was sequenced at Nam Khoa (Vietnam) company.

Table 1

The sequences of primers

	Sequence (5'-3')	Length of product (bps)	Reference
SPS-F	AGTTCGTTTCTTCGTTTGGCT	198	Schemberger et al. (2020)
SPS-R	TTGGCGCTTCTTTTGTGATGG		

Note: F: Forward, R: Reverse

3. Results and discussion

The concentration as well as purity of cDNA, which were totally converted according to the guidelines of SensiFAST cDNA Synthesis Kit, were evaluated by the method of OD. The values of OD of cDNA are listed in Table 2. The purity of cDNA and RNA is determined by evaluating the ratio of absorbance at 260nm and 280nm, 260nm and 230nm. As a result, the ratio of 260nm and 280nm, 260nm and 230nm were in the range of 1.8 - 2.0, and 2.0 - 2.2, respectively. It was generally accepted as "pure" for cDNA (Sambrook & Russell, 2001). Based on the value of concentration, the samples of cDNA were adjusted to 200ng to carry out the assay of Real-time PCR.

Table 2

The value of OD

	OD			**Ratio**		**Concentration (µg/mL)**
	230nm	260nm	280mm	260/280	260/230	
Exocarp	0.618	1.345	0.716	1.878	2.176	67.250
Mesocarp	0.593	1.267	0.642	1.974	2.137	63.350

Real-time PCR was applied to evaluate the expression of *SPS2* as the thermal protocol described above. The results of Real-time PCR showed the positive expression of *SPS2* in both samples of exocarp and mesocarp with the Ct of 27.19, and 28.12, respectively, as showed in Figure 1.

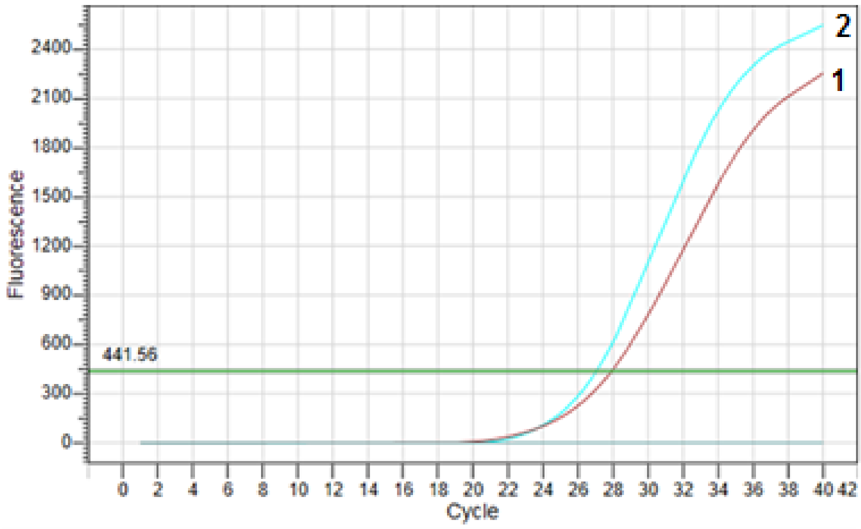


Figure 1. The amplification plot of the gene of *SPS2*
1. Exocarp; 2. Mesocarp tissue

For the authenticity, the electrophoresis and DNA sequencing were conducted. The result of electrophoresis was consistent with the Real-time PCR, showing a distinct clear band with a size of 198 bps, as illustrated in Figure 2. The results of sequencing indicated that both the forward strand and reverse strand displayed clear reading: single peaks, as showed in Figure 3. Additionally, a BLAST analysis was conducted, revealing a sequence similarity with *Cucumis melo* L. (accession number: LN713258.1) in the NCBI database, with a similarity of 96.32%, 96.00% for the forward strand and reverse strand, respectively. Therefore, they concluded that the currently established protocol could be applied for the evaluation of *SPS2* in *Cucumis melo* L. Indeed, the establishment of this procedure is crucial for assessing the expression of the *SPS2* gene in Golden Melon fruits at different stages, which are associated with the accumulation of sucrose, the primary determinant of the fruit's sweetness. By evaluating the gene expression at various stages, researchers can gain insights into the regulatory mechanisms and factors influencing sucrose accumulation in Golden Melon fruits, ultimately contributing to a better understanding of the fruit's sweetness development and quality.

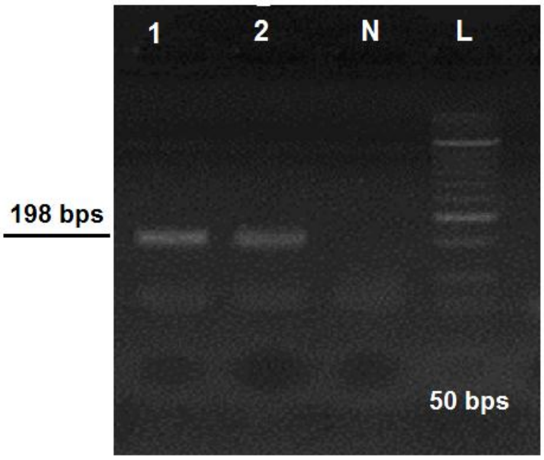
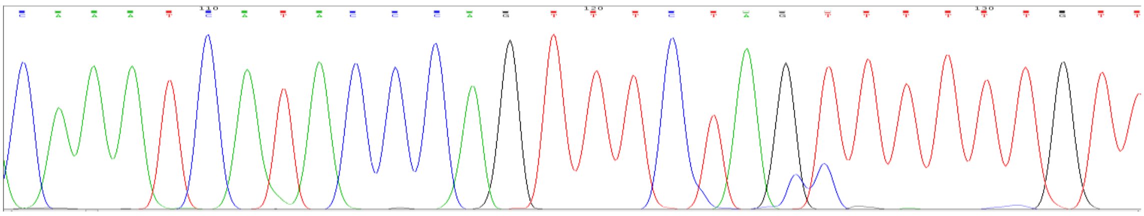
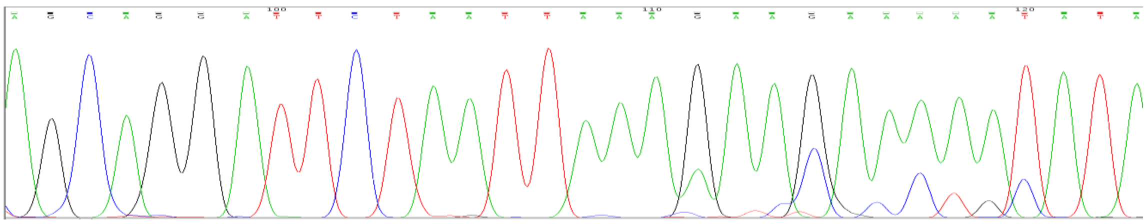


Figure 2. The result of electrophoresis analysis of *SPS2*
1. exocarp; 2. mesocarp tissue; N: negative; L: 50-bps ladder



(A)



(B)

Figure 3. The sequencing of *SPS2*
(A): Forward strand; (B) Reverse strand

4. Conclusion

We successfully established the protocol of Real-time PCR for evaluating the expression of the gene of *SPS2* in golden melon. The successfully established protocol consists of the following steps: RNA extraction by TRIzol (pH = 4), cDNA conversion by SensiFAST cDNA Synthesis Kit, and the real-time PCR assay with a set of primers of SPS-F and SPS-R.

Declaration of Interest

The authors declare no conflicts of interest.

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