Scientia Horticulturae 220 (2017) 259–266

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Ectopic expression of apple *MdSUT2* gene influences development and abiotic stress resistance in tomato

Qi-Jun Ma[a,1], Mei-Hong Sun[a,1], Jing Lu[a], Xiao-Ping Zhu[a], Wen-Sheng Gao[b,*], Yu-Jin Hao[a,*]

[a] National Key Laboratory of Crop Biology, National Research Center for Apple Engineering and Technology, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai-An, Shandong 271018, China
[b] Shandong Fruit and Tea Technology Services, Jinan, Shandong 250013, China

ARTICLE INFO

Keywords:
Apple sucrose transporter MdSUT2
Ectopic expression
Abiotic stress
Early flowering
Fruit ripening
Tomato

ABSTRACT

Sucrose transporters are crucial factor in carbon partitioning and in response to environmental stresses. They coordinate sucrose transport with plant growth and development. In this study, an apple sucrose transporter gene *MdSUT2* was ectopically transformed into tomato. Transgenic tomato lines were used for functional characterization. Soluble sugar content was increased in transgenic tomato lines. Esculin uptake assay indicated sucrose transport activity was improved in the roots of transgenic tomato lines. Meanwhile, the *MdSUT2* transgenic lines accumulated more anthocyanin in leaves when grew under low temperature conditions than the WT control, and noticeably enhanced tolerance to drought and salt stresses. The ectopic expression of *MdSUT2* resulted in early flowering and fruit-ripening in the transgenic tomatoes. These findings provide evidence for the role of sucrose transporters in promoting fruit ripening and resistance to abiotic stresses.

1. Introduction

Fruit ripening is often accompanied by a large accumulation of carbohydrates that provide energy during fruit development (Rolland et al., 2002). Sucrose is the major form of carbohydrates for long distances transportation in most plants. Much investigation has been performed on the subject of source to sink linkage via the phloem in recent years (Williams et al., 2000). Comparing to the poor understanding of the process of phloem unloading into sink tissues such as roots, seeds and tubers, how sugars loading into the phloem from source tissues, primarily leaves, has been well characterized (Griffiths et al., 2016).

Sucrose transporters have been suggested to play a vital role in carbon metabolism and development in fruit (Jia et al., 2015). So, it is essential to identify sucrose transporters function in the process of fruit ripening. Sucrose transporters (SUCs or SUTs) are central machineries to export sucrose from source leaves to sink tissues. Sucrose transporters, belonging to different clades (SUT1-SUT5), show different tissue specificity, substrate affinity and specificity, and subcellular localization, reflecting distinct physiological functions in plants. SUT1 represents the high-affinity sucrose proton co-transporter while SUT4 is a low-affinity transporter (Sun et al., 2008). Meanwhile, SUT2 is suggested to differ structurally from the other SUTs. It is characterized with an extended N-terminal, an elongated central cytoplasmic loop, and a slightly shorter C-terminus than that of clade 1 SUT proteins (Kühn and Grof, 2010).

In *Arabidopsis*, three distinct subfamilies (type SUT1, SUT2 and SUT4) contain a total of nine SUC transporters (Aoki et al., 2006). AtSUC1, AtSUC2, AtSUC5, AtSUC6, AtSUC7, AtSUC8 and AtSUC9 belong to the high-affinity dicot SUT1 clade (Kühn and Grof, 2010). AtSUC4 is the SUT4 clade of low-affinity sucrose transporter. AtSUC3 is the SUT2 clade type transporter (Meyer et al., 2000). The special function of each SUC subfamily is determined by their location (Stadler et al., 1999). For example, *AtSUC2* is responsible for phloem loading of sucrose in source leave (Gottwald et al., 2000; Chandran et al., 2003).

Besides as the major important transported carbohydrate from the leaves to the fruit, sucrose may also act as a non-nutritive regulator of cellular metabolism, possibly by altering gene expression (Rolland et al., 2002; Vaughn et al., 2002; Ruan et al., 2010). Therefore, sucrose transporters (SUTs) play a vital role in the regulation of plant growth and development. It is reported inhibition of the expression potato sucrose transporter StSUT4, mainly expressed in sink organs including tubers, sink leaves and flowers, leads to early flowering and increased tuber yield (Chincinska et al., 2008). Sucrose transporters are the keys

Abbreviations: HPLC, High Performance Liquid Chromatography; MDA, malondialdehyde; SUTs, Suc transporters; TBA, thiobarbituric acid; TRV, tobacco rattle virus; WT, wild-type

* Corresponding authors.
E-mail addresses: 13906413476@163.com (W.-S. Gao), haoyujin@sdau.edu.cn (Y.-J. Hao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scienta.2017.04.013
Received 26 January 2017; Received in revised form 6 April 2017; Accepted 7 April 2017
Available online 23 April 2017
0304-4238/

in resource-sink allocation system. Understanding the regulation mechanism will assist in elucidating the growth and quality of the fruit.

Generally, environmental stresses affect sucrose supply by inhibiting photosynthesis (Gupta and Kaur, 2005; Ruan et al., 2010; Savage et al., 2015). Hence, cross-membrane transport and the distribution of sugars throughout the plant are central steps in plant stress resistance (Lemoine et al., 2013). In higher plants, *SUCs* genes are also involved in the response to various abiotic stresses such as high salinity, drought and low temperatures. *AtSUC1* and *AtSUC2* transcripts are up-regulated in response to low temperature (Lundmark et al., 2006). In rice, only *OsSUT2* from five rice *SUC* genes is up-regulated under drought and salinity stresses (Ibraheem et al., 2011). AgSUT1 is a high affinity H^+/sucrose transporter in *Celery*. Its transcript level decreased in all tissues upon treated with high salinity, suggesting its associatioin with salt tolerance (Noiraud et al., 2000).

Here, we characterized the functions of *MdSUT2* gene in abiotic stress response and the regulation of plant growth and development in tomato. *MdSUT2* overexpressing lines showed improved resistance to low temperature, drought and salt stress. Moreover, overexpression *MdSUT2* gene affects the growth and development process and lead to early ripening of tomato fruit.

2. Materials and methods

2.1. Plant material, plasmid construction, transformation of tomato and stress treatments

For normal soil growth, tomato plantlets were transferred to pots containing a mixture of soil/perlite (1:1) and grown in the greenhouse under a 16 h/8 h light/dark and 25 °C day/night cycle.

The coding region of *MdSUT2* was amplified from apple total RNA using an RT-PCR system. The primers used were forward (5′-AGTCGCGGCCATTACTGAGTC-3′) and reverse (5′-TCCCCCAAATAAA GCATCCCATG-3′). To enable ectopic expression of *MdSUT2* in tomato, the cDNA for this gene was linked to the 35S promoter for constitutive expression. Plasmids were introduced into *Agrobacterium tumefaciens LBA4404*. The three independent lines were used for subsequent analysis. The transgenic plants and wild type were grown for 3 weeks under normal conditions. Then they were treated with drought, salt and low temperature.

T2 generation of transgenic tomato (*Solanum lycopersicum* M. cv. Tianjinbaiguo) were used in the investigation. For the salt tolerance assay, 3-week-old plants were flooded for with NaCl solution every 3 days, incrementally increasing with each successive watering from 100 to 150, 200, and 250 to a final concentration of 300 mM NaCl. For the drought assay, water was withheld from 3-week-old tomato plants in a growth chamber for 2 weeks. For low temperature stress, seedlings of one-week-old tomato was grown under 4 °C condition for 2 days.

2.2. RNA extraction, RT-PCR and qRT-PCR assays

Total RNA was extracted from WT and transgenic tomato for semi-quantitative and real-time reverse transcription PCR analysis. For real-time reverse transcription PCR analysis, the reactions were performed using iQ5 system (Bio-Rad, Hercules, CA, USA) according to the manufacture's instructions. For all analyses, the signal obtained for a gene of interest was normalized against the signal obtained for *Actin1* gene. Three biological replicates of each sample were assayed. The relative quantification of specific mRNA levels was calculated from the cycle threshold (Ct) using the 2^{-DDCt} method.

For semi-quantitative RT-PCR, the reactions were conducted according to the manufacturer's instructions (TransGen, Beijing, China) using the following cycling parameters: pre-incubation at 95 °C for 5 min, followed by 32 cycles of 95 °C (20 s), 58 °C (20 s), and 72 °C (20 s), with a final extension at 72 °C for 10 min. Primers used in table S1.

2.3. Western blot assays

Proteins were extracted from tomato and fractionated on a 12% SDS-PAGE gel and electrotransferred to an Immobilon-P PVDF membrane (Millipore). The membranes were blocked at 4 °C for 12 h with blocking solution. Immunoblots were performed at 4 °C overnight with affinity-purified mouse polyclonal anti-Myc. The membranes were washed with PBS-Tween (5 × 5 min) and treated with the corresponding secondary antibodies (Abmart) (1:20000 dilutions) conjugated to horseradish peroxidase. Immunoreactive bands were visualized using a chemiluminescence detection kit (SuperSignal®, Pierce Biotechnology).

2.4. Soluble sugar content

Sugars were extracted from the WT and transgenic plants. 1 g (FW) of samples was homogenized with 2 ml of 80% ethanol solution in a mortar and pestle. After heating the homogenate in a water bath at 75 °C for 10 min, the insoluble residue was removed by centrifuging at 5000 g for 10 min the precipitate was re-extracted with 2 ml of 80% ethanol at 75 °C and recentrifuged. The supernatants were pooled and dried under a stream of hot air, and the residue was resuspended in 1 ml of water and desalted through a column of ion-exchange resin. The filtrate was used for soluble sugar determinations by HPLC.

2.5. Ethylene measurements

Ethylene production was determined. Fruit were harvested at different ripening stages, weighed and transferred to 1 l gas-tight jars. The jars were sealed and incubated at 25 °C for 2 h. Then 1 ml of gas sample was withdrawn from the headspace with a syringe and injected into a gas chromatograph (SQ-206, Beijing, China). Three independent samples derived from five fruits at each ripening stage were used for the ethylene measurements.

2.6. The total anthocyanin content

Total anthocyanins were extracted using a methanol-HCl method and detected as described by Hu et al. (2016).

2.7. MDA content

The determination of malondialdehyde (MDA) contents was described in detail. The leaves were cut into several segments in 5 ml of 10% phosphate buffered saline (PBS) and centrifuged at 12000*g* for 10 min. Two milliliters of the supernatant was added to 5 ml of 0.5% thiobarbituric acid (TBA, in 10% TCA), and the reaction mixture was incubated at 100 °C in a water bath for 10 min. The reaction was cooled to room temperature, and the absorbance of the supernatant at 450, 532 and 600 nm was determined using a UV–vis spectrophotometer (UV-2450). Thiobarbituric acid was used as a blank. The following formula was used to estimate MDA levels: MDA content (mmol g^{-1} FW) = [6.542 × (OD532 − OD600) − 0.559 × OD450] (mmol L^{-1})*V (ml)/fresh weight (g FW). Statistical analysis was performed for all of the data.

2.8. Esculin uptake

Plants were grown on plates for 7 d in the light on ½ strength MS media with 30 mM sucrose. Seedlings were transferred to ½ strength MS liquid media with 30 mM sucrose supplemented with 1 mM esculin (Sigma E8250) for 30 min. Seedlings were then rinsed and mounted on glass slides in ½ strength MS liquid media without esculin. The fluorescence was measured using a ZEISS LSM880 spectrofluorometer with a 367 nm excitation wavelength and a 454 nm emission wavelength.