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Research article

Overexpression of sucrose transporter gene *PbSUT2* from *Pyrus bretschneideri*, enhances sucrose content in *Solanum lycopersicum* fruit

Li-Fen Wang [a, b], Xiao-Xiao Qi [a], Xiao-San Huang [a], Lin-Lin Xu [a], Cong Jin [a], Jun Wu [a], Shao-Ling Zhang [a, *]

[a] Center of Pear Engineering & Technology Research, College of Horticulture, Nanjing Agricultural University, Nanjing, 210095, China
[b] Gold Mantis School of Architecture, Soochow University, Suzhou, 215123, China

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ABSTRACT

Sucrose transporters (SUTs) belong to the major facilitator superfamily. The function of SUTs has been intensively investigated in some higher plants, whereas that in pear fruit is unknown. In this study, the cloning and functional characterization of a sucrose transporter, *PbSUT2*, in pear (*Pyrus bretschneideri* Rehd. cv. 'Yali') fruits are reported. *PbSUT2* encoded a protein of 498 amino acid residues, and was localized in the plasma membrane of transformed onion epidermal cells and Arabidopsis protoplasts. Phylogenetic analysis revealed that *PbSUT2* belonged to the SUT4 clade. The phenotype of overexpression of *PbSUT2* tomato plants included early flowering, higher fruit quantity and lower plant height. Overexpression of *PbSUT2* in transgenic tomato plants led to increases in the net photosynthetic rate in leaves and sucrose content in mature fruit compared with wild-type tomato plants, and a decrease in the contents of glucose, fructose and total soluble sugars in mature fruits. These results suggested that *PbSUT2* affected sucrose content in sinks and the flowering phase during tomato plant growth and development.

1. Introduction

Sucrose is the main carbohydrate transported between plant leaves and sink organs. The supply of carbohydrate to sink organs is essential for growth in higher plants (Kühn and Grof, 2010; Lalonde et al., 2004). Sucrose transporters (SUTs) play an important role both in phloem loading in source tissue and sucrose unloading into sink tissue (Rennie and Turgeon, 2009). The first isolated SUT gene, SoSUT1, was from spinach (Riesmeier et al., 1992). Subsequently, SUT genes were isolated from other plant species (Aoki et al., 1999; Sauer et al., 2004; Hackel et al., 2006; Fan et al., 2009; Frost et al., 2012; Berthier et al., 2014).

SUTs have been divided into five independent phylogenetic clades (SUT1-SUT5) in all plants (Kühn and Grof, 2010). The SUT1 clade has the high affinity of dicot-specific SUTs and plays roles in phloem loading or sucrose import into sink cells, which are localized at the plasma membrane (Riesmeier et al., 1994; Slewinski et al., 2009). SUT2 and SUT4 transporters contain both monocot and dicot members; SUT2 clade members are also localized at the plasma membrane, but SUT4 members are located at the plasma membrane or tonoplast (Weise et al., 2000; Endler et al., 2006; Reinders et al., 2008; Payyavula et al., 2011). SUT3 and SUT5 clade members are monocot-specific transporters (Kühn and Grof, 2010), and SUT5 members have not been characterized.

Different members of each SUT clade show different functions. Knockdown, knockout and overexpression of SUT genes provide genetic evidence that sucrose transporters have essential roles in sucrose transport in plants (Leggewie et al., 2003; Hackel et al., 2006; Gould et al., 2012). *Arabidopsis thaliana AtSUC1*, *AtSUC2*, and *AtSUC5-AtSUC9* are members of the dicot SUT1 clade (Kühn and Grof, 2010). *AtSUC2* has a role in retrieving the leaked sucrose in the transport phloem of *Arabidopsis*, but *AtSUC1* cannot significantly retrieve leaked sucrose (Gould et al., 2012). *AtSUC9* can mediate the balance of sucrose distribution (Jia et al., 2015). *SoSUT1*, *StSUT1* and *LeSUT1* are also members of the SUT1 clade; *SoSUT1* in potato (*Solanum tuberosum* L.) increases the sucrose uptake rate in plasma membrane vesicles (Leggewie et al., 2003). In early stages of tuber development, the expression of *StSUT1* reduces which leads to reduced fresh weight accumulation in tubers (Kühn, 2003). Overexpression of *StSTU1* increased in developing cotyledons of

* Corresponding author.
E-mail address: slzhang@njau.edu.cn (S.-L. Zhang).

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transgenic peas (Rosche et al., 2002). Antisense lines of the *LeSUT1* gene did not produce fruits (Hackel et al., 2006). The primal function of SUT2/SUC3 might be as sucrose sensors in sieve elements (Barker et al., 2000; Reinders et al., 2002); however, this conclusion was later debated (Sauer, 2007). For example, *LeSUT2* is directly involved in phloem unloading in tomato fruits, and thereby influences the fruit yield of tomato (*Solanum lycopersicum*) plants (Hackel et al., 2006).

In contrast, the SUT4 clade has a different suggested function to SUT1 (Kühn, 2003). SUT4 promotes sucrose uptake with low affinity and has high transport capacity within the SUT family (Weise et al., 2000; Weschke et al., 2000). Members of the SUT4 clade contain mainly barley *HvSUT2* (Weschke et al., 2000), *Arabidopsis thaliana AtSUC4* (Weise et al., 2000; Hofmann et al., 2009), tomato *LeSUT4* (Weise et al., 2000; Reinders et al., 2002), lotus *LjSUT4* (Reinders et al., 2008), potato *StSUT4* (Weise et al., 2000; Chincinska et al., 2008), tobacco *NtSUT4* (Okubo-Kurihara et al., 2011), poplar *PtaSUT4* (Payyavula et al., 2011) and sweet orange *CsSUT4* (Zheng et al., 2014). *AtSUC4* releases sucrose from the vacuole into the cytoplasm (Schulz et al., 2011). Sucrose content in leaves and stems increases *in PtaSUT4*-RNA interference (RNAi) plants, and phenylpropanoid metabolism is changed (Payyavula et al., 2011); In *PtaSUT4*-RNAi plants of poplar, the leaf area increases and photosynthesis rates decrease under well-watered conditions (Frost et al., 2012). *StSUT4*-RNAi plants have earlier flowering phenotypes and higher tuber production compared to wild-type plants under far-red wavelength conditions (Chincinska et al., 2008). Whereas sucrose export from source leaves is promoted in rice *OsSUT2* plants (Eom et al., 2011). Thus, SUT4 transgenic plants do not show a consistent phenotype in different species.

Sucrose improves fruit flavor and is the most important component for sweet taste in fruit (Róth et al., 2007). Pear is an important fruit in China; however, sucrose content varies greatly in mature fruits of all pear varieties (Yao et al., 2010). Previous research has focused on the relationship of sucrose accumulation with the activity of enzymes involved in sugar metabolism (Hubbard et al., 2006; Zhang et al., 2014), and there are less data concerning the effect of sucrose transporters isolated from pear fruit on sucrose content.

Several functional sucrose transporters have been cloned from fleshy fruits such as grapes (Ageorges et al., 2000), tomato (Hackel et al., 2006), apple (Fan et al., 2009; Peng et al., 2011), sweet orange (Zheng et al., 2014) and pear (Zhang et al., 2013). Although SUT genes have been cloned from diverse fleshy fruits, previous studies gave priority to cDNA cloning, expression analysis, or transport activity. To date, there has been less attention on the effects of overexpressing the sucrose transporter genes isolated from fleshy fruits on the sucrose content in fruits and plant phenotypes. In this study, a new sucrose transporter *PbSUT2* gene was cloned from pear fruit and its function was analyzed using transgenic tomato. The net photosynthetic rate in leaves of transgenic tomato plants changed and the sucrose content increased in their fruit.

2. Results

2.1. Cloning of a full-length cDNA of the PbSUT2 gene and sequence analysis

Polymerase chain reaction (PCR) was carried out with special primers spanning upstream and downstream of the Pbr025968 (*PbSUT2*) open reading frame (ORF). Sequence analysis revealed a 1497-bp ORF for *PbSUT2* that encoded a 498-amino acid protein with a predicted molecular mass of 53.42 kDa and an isoelectric point (*pI*) of 8.96. The basic local alignment search tool (BLAST) searches revealed that the deduced amino acid sequence of *PbSUT2* shared roughly 94%, 76% and 71% identity with homologous sequences from apple (*MdSUT1*, AAR17700), grape (*VvSUT*, AAD55269) and *Arabidopsis thaliana* (*AtSUT4*, AAGO9191), respectively (Fig. 1). The predicted membrane topology of PbSUT2 protein consisted of 12 transmembrane domains (Fig. 2) with both N- and C-termini on the cytoplasmic side, which could be divided into two parts of six hydrophobic loops each. The two parts were separated by a large central hydrophilic segment of about 40 amino acids (Fig. 2). This structure exhibited common characteristics of the members of the major facilitator superfamily (MFS; Marger and Saier, 1993). To compare the amino acid similarity between the predicted PbSUT2 peptides and the SUTs isolated from other plant species, an unrooted phylogenetic tree was constructed using the program MEGA 5.0 (Fig. 3) — the 28 sequences were distributed into five SUT clades: SUT1-SUT5 (Kühn and Grof, 2010). This result showed that *PbSUT2* belonged to the SUT4 clade among sucrose transporters and was most closely related to the functional sucrose transporter *MdSUT1* (AAR17700) from apple fruit (Fig. 3).

2.2. Expression pattern of PbSUT2 in 'Yali' fruits

The expression pattern of *PbSUT2* was determined by real-time quantitative PCR (qRT-PCR). *PbSUT2* gene was expressed throughout fruit development, but was higher before fruit maturity [140 days after full bloom (DAFB)] and had a peak at the fruit rapid enlargement stage (100 DAFB). In contrast, the expression levels were lower in mature fruit (160 DAFB; Fig. 4).

2.3. PbSUT2 located in plasma membrane

To determine the subcellular localization of *PbSUT2* gene, the fusion protein [pCAMBIA1302–PbSUT2–Green Fluorescent Protein (GFP)] was constructed by subcloning the *PbSUT2* ORF without the stop codon into the upstream region of the *GFP* gene in the pCAMBIA1302 vector under the control of the Cauliflower mosaic virus (CaMV) 35S promoter. The subcellular localization of PbSUT2 was determined by monitoring the GFP fluorescence in onion epidermis cells. In epidermal cells transformed with the control vector pCAMBIA1302–GFP, green fluorescence was distributed in both the cytoplasm and nucleus of epidermal onion cells (Fig. 5A–C). In contrast, green fluorescence was exclusively detected in the plasma membrane of cells transformed with the fusion plasmid of pCAMBIA1302–PbSUT2–GFP (Fig. 5D–F). The results showed clearly that PbSUT2 localized to the plasma membrane (Fig. 5), consistent with results from the localization assays of Arabidopsis protoplasts (Suppl. Fig. 1).

2.4. Phenotypic analysis of transgenic plants

The expression level of *PbSUT2* in red tomato fruits of overexpressing plants was measured by semi-quantitative PCR. *PbSUT2* expression was detected in lines 45 and 62, but not in fruit of wild-type (WT) plants (Fig. 6). *PbSUT2* overexpressing transgenic tomato plants showed some morphological modifications after 9 weeks of growth, they started to flower an average of 11 d before WT (Figs. 7A, B and 8A). *PbSUT2* overexpressing plants had lower plant height throughout their development compared to WT; and their height was significantly reduced compared to WT plants at the mature fruit stage (Fig. 8B). However, the number of fruit of *PbSUT2*-overexpressing plants increased significantly compared with that of WT plants at the mature fruit period (Figs. 7C and 8C).