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Dwarfing Rootstock 'Yunnan' Quince Promoted Fruit Sugar Accumulation by Influencing Assimilate Flow and PbSWEET6 in Pear Scion

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Abstract: 'Yunnan' quince (*Cydonia oblonga* Mill.) is used as the dwarfing rootstock for pear (*Pyrus* spp.). Here, we reported that the sugar contents in mature 'Zaosu' pear fruit grafted on 'Yunan' quince (Z/Q) were higher than that in 'Zaosu' pear fruit grafted on 'Duli' (*Pyrus betulifolia*) (Z/D). To investigate the underlying mechanism, the leaf photosynthetic capacity and the leaf-to-fruit assimilate transport capacity were initially analyzed. The leaf photosynthetic capacity was similar between Z/Q and Z/D, but the assimilate transport capacity was greater for Z/Q than for Z/D. Sugar transporters mediate the distribution of assimilates; therefore, changes in *PbSWEET* transcriptional patterns were examined. *PbSWEET6* was highly expressed in Z/Q fruit. Thus, the *PbSWEET6* function related to assimilate transport was further verified. Sucrose and glucose contents increased in transgenic tomato fruit and pear fruit calli overexpressing *PbSWEET6*. Taken together, these results suggest that 'Yunnan' quince positively regulated fruit sugar contents by influencing the flow of PbSWEET6-involved assimilates in the scion.

Keywords: graft; *PbSWEET6*; sugar transport; 'Zaosu' pear; *Cydonia oblonga*

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1. Introduction

Dwarfing rootstocks are used for the production of dwarf fruit trees in modern cultivation systems, which is consistent with the changes in pear cultivation technology toward dwarfing and dense planting [1–3]. Dwarfing rootstocks affect scion growth and are important for improving fruit quality and yield [4,5]. The combination of quince as the base stock and 'Hardy' (*Pyrus communis*) as the interstock leads to a better dwarfing effect and significantly improves the quality of the scion fruit [6]. The fruit sugar content is influenced by photosynthesis, but it is also affected by the transport and accumulation of assimilates, with sugar transporters playing an essential role in this process [7–9].

Sugar transporters are mainly divided into the following three superfamilies: the major facilitator superfamily, the sodium solute symporter family, and the Sugar Will Eventually be Exported Transporter (SWEET) family [10,11]. The SWEET family, which was identified relatively recently, helps to mediate the influx and efflux of sugars across the plasma membrane [12]. Additionally, SWEETs can be divided into four phylogenetic clades (Clades I–IV) [13], of which Clades I and II consist mainly of hexose transporters, whereas Clade III comprises sucrose transporters and Clade IV includes fructose transporters [14,15]. The diversity in the SWEET genes in plants enables the encoded proteins to function in various developmental and physiological processes. In *Arabidopsis thaliana*, AtSWEET1 is a low-affinity glucose transporter that contributes to glucose uptake and efflux [16]. Mutations to both *AtSWEET11* and *AtSWEET12* reportedly decrease leaf assimilate exudation and significantly inhibit sucrose loading and plant growth, resulting in the accumulation

of sugar and starch in source leaves [17]. Similar findings have been reported for *Zea mays* [18]. Moreover, *AtSWEET17* and *AtSWEET16*, which encode vacuolar sugar transporters, have important functions associated with plant growth and development [19–21]. In tomato, *SlSWEET12c* may promote sucrose unloading from phloem during the tomato fruit development stage [22]. Moreover, *SlSWEET7a* and *SlSWEET14* encode hexose and sucrose transporters, and the silencing of these genes results in increases in plant height and fruit size [23]. The overexpression of *VvSWEET10* in grape calli and tomato significantly increases the glucose, fructose, and total sugar levels [24]. Previous research revealed 18 SWEETs in pear [25]. In 'Nanguo' pear (*Pyrus ussuriensis*), PuSWEET15 increases the fruit sucrose content [26].

Sugar transport and accumulation substantially affect fruit sweetness [24,27]. Different types of rootstocks can influence the transport of sugars into the scion fruit [28]. SWEETs play key roles in the accumulation of sugars in fruit [23–25]. However, the mechanism by which dwarfing rootstocks affect sugar transport in scion fruit and related sugar transporters is still unclear. In this study, 'Zaosu' (*Pyrus bretschneideri* Rehd.) pear grafted on 'Yunnan' quince (*Cydonia oblonga* Mill.) and 'Duli' (*Pyrus betulifolia*) were used as the experimental materials. A series of experiments showed that the sugar-transport-related gene *PbSWEET6* was important for mediating the effect of 'Yunnan' quince on 'Zaosu' pear fruit sugar accumulation. The results of this study may be useful for clarifying the mechanism underlying the influence of dwarfing rootstocks on fruit sugar transport in pear.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Twelve-year-old 'Zaosu' pears were grafted on 'Yunnan' quince (Z/Q) using 'Hardy' (*Pyrus communis*) as interstock. Twelve-year-old 'Zaosu' pears were also directly grafted on 'Duli' (Z/D). Z/Q and Z/D trees were cultured in an experimental pear orchard in Meixian, Shaanxi, China (34.28° N, 108.76° E). The soil of the orchard was loam. The orchard adopted conventional management, and the management level was consistent. Mature fruits, leaves, carpopodium, and phloem of fruiting branches were harvested at 110 DAFB (days after flower bloom) in 2021. Thirty fruits from each combination were divided into three groups and brought back to the laboratory for fruit quality determination. The flesh of those fruits was immediately frozen in liquid nitrogen and stored at −80 °C. Tomato (*Solanum lycopersicum* L., 'Micro-Tom') seeds and cultured fruit calli of 'Starkrimson' pear (*Pyrus communis*) were used for genetic transformation. Tomato plants were grown in a light incubator with a 16 h/8 h (light/dark) photoperiod at 25 °C.

2.2. Photosynthetic Capacity and Assimilate Distribution

The experiment was carried out at a distance of about 1.5 m from the ground of the plant, and the biennial fruiting branch group with one fruit was selected for ^{13}C pulse labeling [29,30]. The experiment began at 9:00 a.m., when 2 mol·L^{-1} HCl solution was injected into a plastic bottle containing 1 g of $Ba^{13}CO_3$ (98%; Shanghai Research Institute of Chemical Industry, Shanghai, China) with a syringe (the HCl solution was excessive to ensure the complete reaction of $Ba^{13}CO_3$). The processing time was 7 h, during which the bag was gently shaken. At the same time, three plants not contaminated with ^{13}C were selected as blank controls (for the determination of natural ^{13}C abundance). After 24 h, the leaves, fruits, phloem, and xylem of branches were harvested for ^{13}C determination. The samples were dried for 30 min at 105 °C and then dried at 65 °C until reaching constant weight. After grinding, the samples were screened through 100 mesh and mixed well. The ^{13}C abundance of the above samples was determined with an isotope mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Bremen, Germany).

Eight of each combination were randomly selected for photosynthesis measurement. For each tree, five mature leaves were selected from the middle of the bearing branches on the sunny side for determination. Leaf photosynthesis was measured from 9:30 to 11:00 a.m. using the LI-COR 6800 portable photosynthesis system (LI-COR, Huntington Beach, CA, USA). During the measurement, the light intensity was set at 300 and 1000 μmol m^{-2} s^{-1} (90%: 10% red: blue light), the CO_2 concentration in the leaf chamber was set at 400 umol mol^{-1} by a CO_2 mixer, the relative humidity of 60%, the leaf-to-air vapor pressure deficit was kept at 0.1 kPa, and the flow rate was 500 μmol s^{-1}. Then, 3–5 min was allowed for settling in the leaf chamber, and values were recorded after the leaf reached a steady state. The leaves were immersed in a mixed solution of 80% acetone and 95% ethanol (1:1) for 24 h in the dark to extract the chlorophyll and carotenoid, and the spectrophotometric values were obtained at 663 nm, 645 nm, and 440 nm. The corresponding chlorophyll and carotenoid contents were then calculated.

2.3. Measurements of Soluble Solids and Sugar Contents

Thirty fruits were selected from each combination, and every ten fruits were divided into one group for fruit quality determination. The content of soluble solid (Brix%, grams of soluble solid per 100 g of water) in the filtrate was determined with a refractometer (PAL-1, ATAGO, Tokyo, Japan), and titratable acid (%, percentage of mass) was measured with a fruit acidity tester (GMK-835F, G-won Hitech, Seoul, Korea). The components of soluble sugar were measured by GC–MS (ISQ & TRACE ISQ, Thermo Scientific, Waltham, MA, USA). The general steps were as follows: the sample was ground into powder in liquid nitrogen, 0.1 g of the sample was mixed with 1.4 mL of 80% (*v*/*v*) chromatographic methanol, ribitol was added (4 mg/mL) as an internal standard, and the mixture was shocked in a metal bath at 70 °C for 30 min. After centrifugation at 13,000× *g* for 15 min, the supernatant was taken. After that, 750 mL of chromatographic $CHCl_3$ and 1400 mL ddH_2O were added and mixed, then centrifuged at 2200× *g* for 15 min, and 20 μL was taken for vacuum concentration and drying. Finally, it was derivatized with methoxyamine hydrochloride and N-methyl-N-trimethylsilyl-trifluoroacetamide, then stored with brown bottles for subsequent determination.

2.4. Sequence Analysis of PbSWEET6

The sequences of SWEET gene family members were downloaded from the pear genome database (http://peargenome.njau.edu.cn, accessed on 10 December 2021) and named in reference to Li [25]. The SWEETs protein sequences of *Arabidopsis thaliana* were downloaded from Phytozome v13 (https://phytozome-next.jgi.doe.gov/, accessed on 10 December 2021), and *Solanum lycopersicum* and *Vitis vinifera*, searched on the NCBI database (https://www.ncbi.nlm.nih.gov/, accessed on 10 December 2021), were used to construct a phylogenetic tree with the maximum likelihood method and a bootstrap analysis was performed with MEGA X software. Bootstrap values were calculated from 1000 replicate analyses.

The protein accessions used were as follows: AtSWEET1 (*Arabidopsis thaliana*, AT1G21460.1), AtSWEET2 (AT3G14770.1), AtSWEET3 (AT5G53190.1), AtSWEET4 (AT3G28007.1), AtSWEET5 (AT5G62850.1), AtSWEET6 (AT1G66770.1), AtSWEET7 (AT4G10850.1), AtSWEET8 (AT5G40260.1), AtSWEET9 (AT2G39060.1), AtSWEET10 (AT5G50790.1), AtSWEET11 (AT3G48740.1), AtSWEET12 (AT5G23660.1), AtSWEET13 (AT5G50800.1), AtSWEET14 (AT4G25010.1), AtSWEET15 (AT5G13170.1), AtSWEET16 (AT3G16690.1), AtSWEET17 (AT4G15920.1); PbSWEET2 (*Pyrus bretschneideri*, XP_018501061.1), PbSWEET5 (XP_009337540.1), PbSWEET6 (XP_009349481.1), PbSWEET8 (XP_009347912.1), PbSWEET9 (XP_009340984.1), PbSWEET10 (XP_009360715.1), PbSWEET12 (XP_009376722.1), PbSWEET13 (XP_009352052.1), PbSWEET14 (XP_009377708.1), PbSWEET18 (XP_009360807.1); PuSWEET15 (*Pyrus ussuriensis*, QIJ69897.1); SlSWEET1-like (*Solanum lycopersicum*, XP_004237722.1), SlSWEET7a (XP_004245483.1), SlSWEET12c (XP_004247459.1), SlSWEET14 (XP_004235340.1); OsS-

WEET11 (*Oryza sativa*, XP_015648423.1), OsSWEET14 (XP_015615538.1), and OsSWEET15 (XP_015623673.1); VvSWEET7 (*Vitis vinifera*, XP_002263697.1), and VvSWEET10 (XP_002284244.1).

2.5. Gene Cloning and Quantitative Real-Time PCR

The total RNA was extracted from the fruits, leaves, phloem, and carpopodium of Z/D and Z/Q using an RNAprep Pure Plant Kit (TIANGEN, Beijing, China). The first-strand cDNA was synthesized from 1 μg of total RNA using the *Evo M-MLV* RT Mix Kit with gDNA Clean for qPCR (AG, Hunan, China).

The qRT-PCR was measured using the Hieff® qPCR SYBR® Green Master Mix (Yeasen, Shanghai, China) on the StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Thermo Fisher Scientific, Albany, NY, USA). The primers of selected genes and *PbActin* (an internal control) were designed on the NCBI and are listed in Table S1. At least three biological replicates were performed and analyzed using the cycle threshold ($2^{-\Delta\Delta Ct}$) method.

2.6. Subcellular Location of PbSWEET6

The full-length coding sequence (CDS) of *PbSWEET6* was amplified and cloned into the pCAMBIA2300 vector fused with the GFP reporter and driven by the CaMV35S promoter. Primers are listed in Table S1. The recombinant plasmid was transformed into tobacco leaves by injection and the empty vector expressing untargeted GFP was used as a control. The GFP green fluorescence was observed with a fluorescence microscope (BX63, OLYMPUS, Tokyo, Japan).

2.7. Agrobacterium-Mediated Tomato and Pear Fruit Calli Transformation

The full CDS of *PbSWEET6* was cloned into the gateway entry vector (pDONR222) and subsequently transferred into the pK7-203 destination vector using LR Clonase II enzymes (Invitrogen, Gibco, Grand Island, NY, USA). The transformation methods of tomato and pear fruit calli were in accordance with Zhang et al. [31].

2.8. Statistical Analysis

The data are presented as the means ± SDs (standard deviations). Significance tests were carried out using SPSS software based on Student's *t* tests at $p < 0.01$ or $p < 0.05$.

3. Results

3.1. 'Yunnan' Quince Increased the Sugar Content of Scion Fruit

The quality of Z/Q and Z/D fruits was determined after harvest at 110 DAFB. The Z/Q fruit was heavier than the Z/D fruit. Moreover, the soluble solid content (SSC) was higher in Z/Q fruit than in Z/D fruit, but there was no significant difference in the titratable acid content (Figure 1A–D). The analysis of sugar contents indicated that fructose, glucose, and sucrose contents were significantly higher in Z/Q fruit than in Z/D fruit (Figure 1E). Thus, 'Yunnan' quince increased the scion fruit sugar content.

3.2. 'Yunnan' Quince Promoted the Transport of Photoassimilates to Fruit

Fruit quality is closely related to photosynthesis. Therefore, the photosynthetic rate and chlorophyll content were analyzed. The data showed no significant difference in the photosynthetic rate between Z/Q and Z/D leaves, implying that there were no differences between 'Yunnan' quince and 'Duli' in terms of their effects on the 'Zaosu' pear photosynthetic rate (Figure 2A). There were also no differences in the chlorophyll a, chlorophyll b, and carotenoid contents between Z/Q and Z/D leaves (Figure 2B–D). The distribution of photoassimilates was determined on the basis of a ^{13}C feeding assay. After 1 day of ^{13}C feeding, the percentage of ^{13}C was higher in Z/Q fruit than in Z/D fruit (Figure 2E). Therefore, 'Yunnan' quince appeared to promote the transport of photoassimilates in the scion.

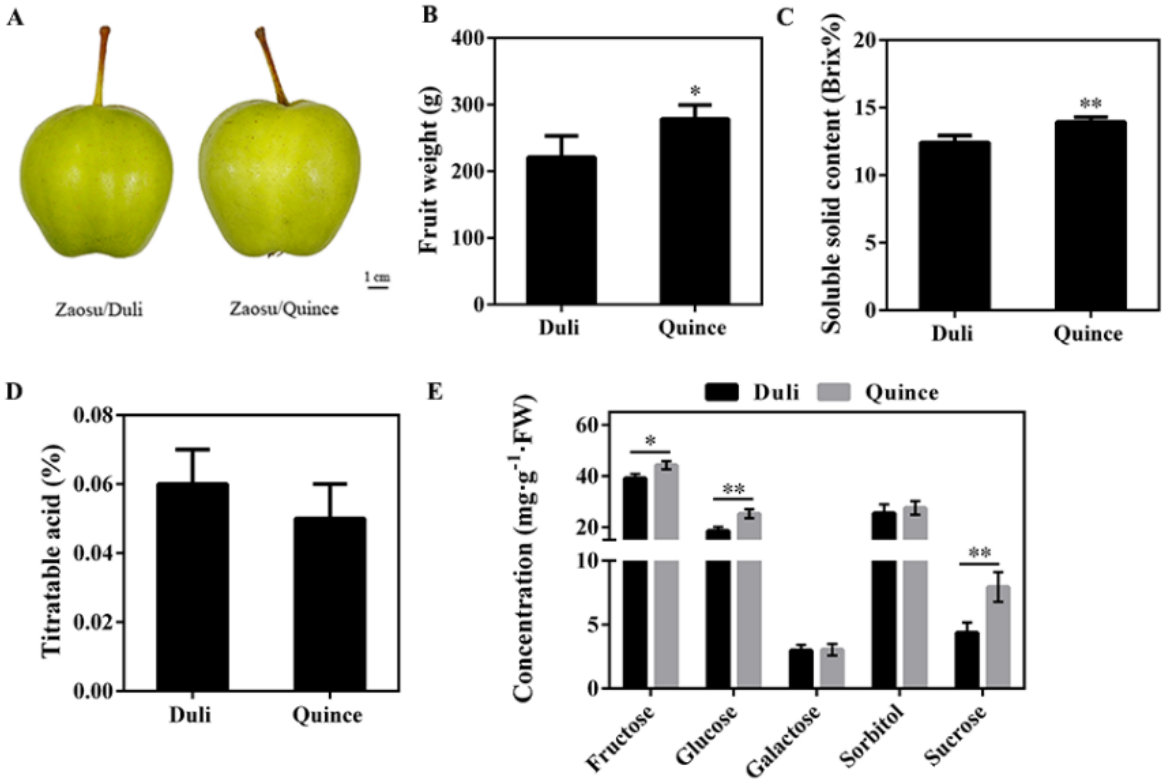

Figure 1. The fruit quality of 'Zaosu' pear grafted on 'Yunnan' quince (Z/Q) and 'Duli' (Z/D). (**A**) Z/Q and Z/D fruits at 110 (DAFB). (**B**) Fruit weight. (**C**) Soluble solid content. (**D**) Titratable acid. (**E**) Concentrations of fructose, glucose, galactose, sorbitol, and sucrose in mature Z/Q and Z/D fruits. The data represent mean values ± SDs. Asterisks indicate significant differences as determined by Student's *t* tests (* $p < 0.05$; ** $p < 0.01$).

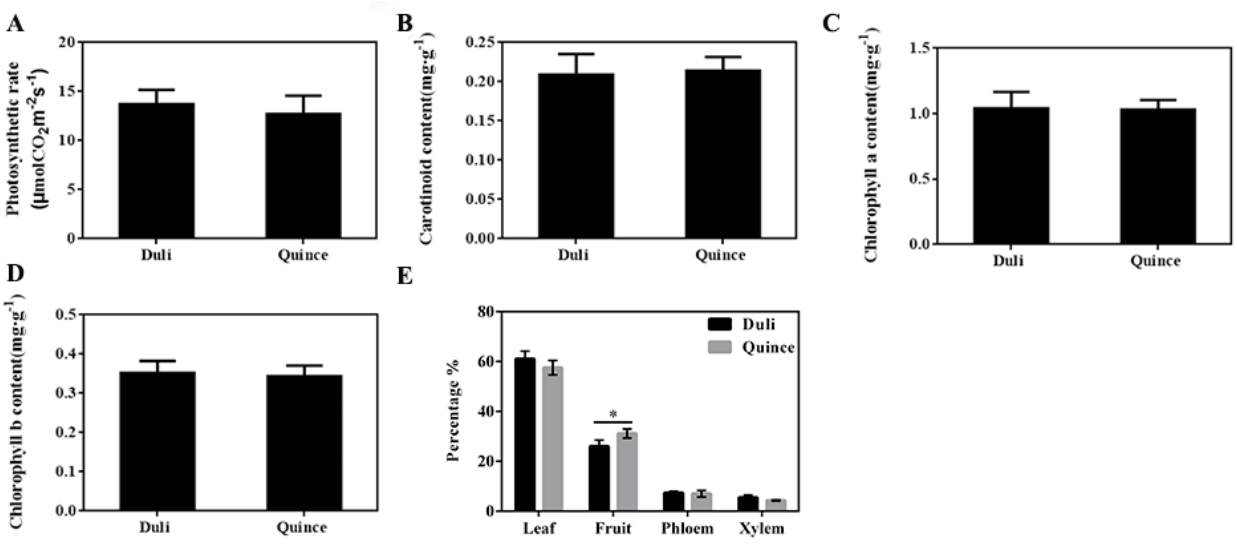

Figure 2. Photosynthetic related indexes and ^{13}C feeding experiment. (**A**) The leaf photosynthetic rate of 'Zaosu' pear grafted on 'Yunnan' quince (Z/Q) and 'Duli' (Z/D). (**B**–**D**) The contents of carotenoid, chlorophyll a, and chlorophyll b. (**E**) The percentage of ^{13}C in each organization. The data represent the mean values ± SDs. Asterisks indicate significant differences as determined by Student's *t* tests (* $p < 0.05$).

3.3. PbSWEET6 May Participate in the Transport of Photoassimilates

Photoassimilates are mainly transported in plants as sugars. A quantitative real-time PCR (qRT-PCR) analysis of 18 *PbSWEET* genes in Z/Q and Z/D fruits showed that *PbSWEET15* and *PbSWEET17* were not expressed in the fruit. The *PbSWEET6*, *PbSWEET8*, *PbSWEET10*, and *PbSWEET14* expression levels were significantly higher in Z/Q fruit than in Z/D fruit. Of these genes, the greatest difference in expression between Z/Q and Z/D fruits was detected for *PbSWEET6* (Figure 3A). Therefore, we focused on *PbSWEET6* in the subsequent analyses. An examination of tissue-specific expression indicated that *PbSWEET6* was highly expressed in the carpopodium, leaf, and fruit (Figure 3B).

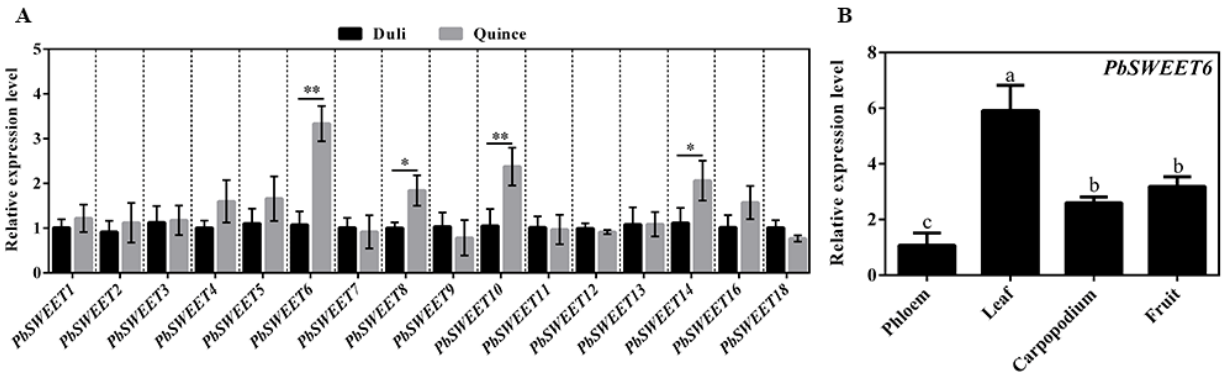

Figure 3. Selected and tissue-specific expression analysis of *PbSWEET6*. (**A**) Relative expression of *PbSWEET* genes from mature fruits of 'Zaosu' pear grafted on 'Yunnan' quince (Z/Q) and 'Duli' (Z/D) as determined by qRT-PCR. (**B**) The relative expression levels of *PbSWEET6* were detected in the phloem, leaves, carpopodium, and fruits of Z/Q during the mature fruit period. The data represent the mean values ± SDs. Different letters represent significant differences (Tukey's HSD, $p < 0.05$) and asterisks indicate significant differences as determined by Student's *t* tests (* $p < 0.05$, ** $p < 0.01$).

3.4. Phylogenetic Analysis and Subcellular Localization of PbSWEET6

MEGA X was used to construct a phylogenetic tree according to the maximum likelihood method and a bootstrap analysis (1000 replicates). The phylogenetic analysis revealed that PbSWEET6 is most similar to PuSWEET15 and AtSWEET15 (Figure 4A), suggesting that it is likely involved in sucrose transport. To determine the precise subcellular location of the PbSWEET6 protein, the *PbSWEET6* CDS was fused to a GFP-encoding sequence. After transforming tobacco plants with this construct, fluorescence was clearly observed near the plasmalemma of cells (Figure 4B).

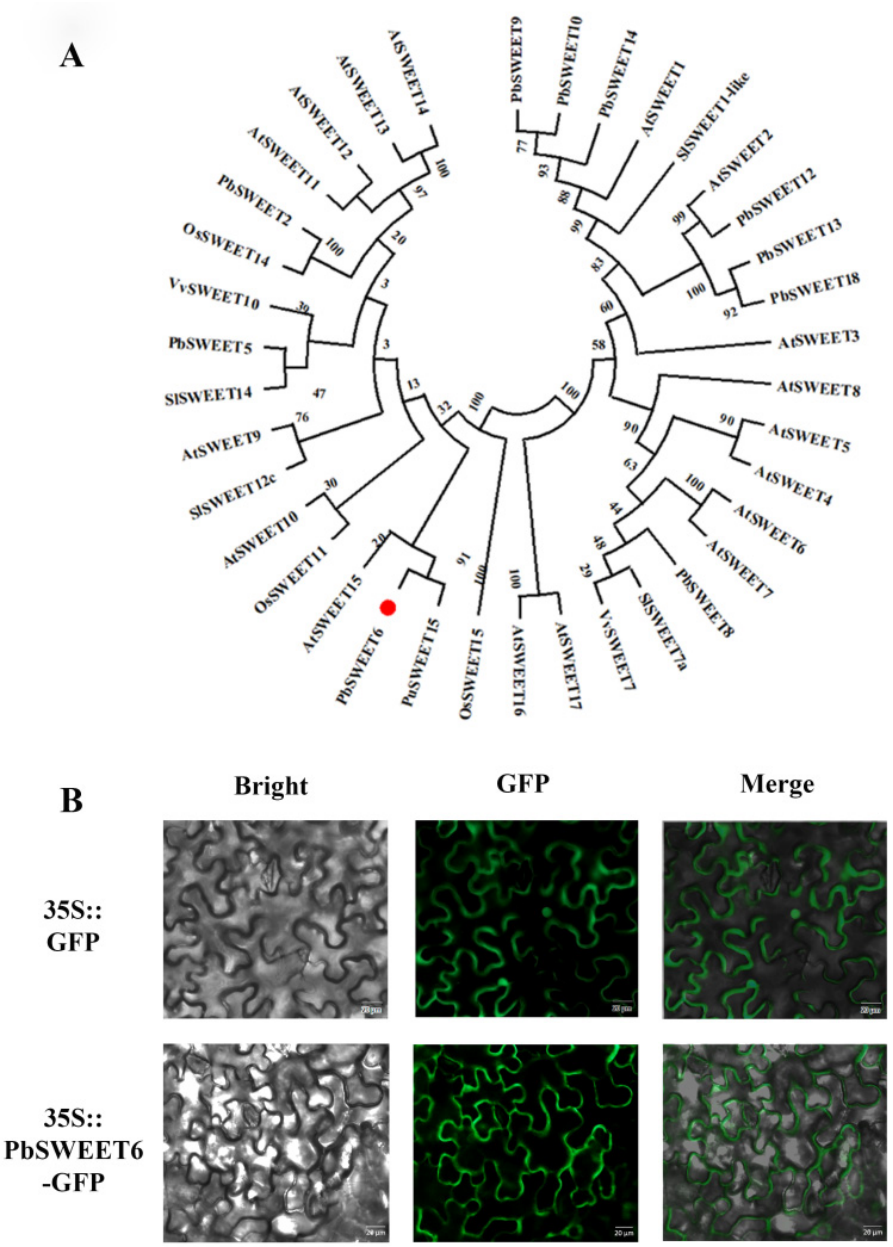

Figure 4. Phylogenetic and subcellular localization analysis of PbSWEET6. (**A**) Phylogenetic analysis of SWEET genes. The phylogenetic tree was inferred using the maximum likelihood method and JTT matrix-based model. PbSWEET6 protein is highlighted with red dots. (**B**) Localization of PbSWEET6. Bar = 20 μm.

3.5. PbSWEET6 Increased the Glucose and Sucrose Content, in Tomato and Pear Fruit Calli

PbSWEET6 was overexpressed in tomato (*PbSWEET6*-OE) to facilitate the further functional characterization of *PbSWEET6*. The PCR and qRT-PCR results confirmed that the tomato plants were successfully transformed. Two transgenic tomato lines were selected for further analyses (Supplemental Figure S1C,D). The overexpression of *PbSWEET6* resulted in significant decreases in plant height and leaf size (Supplemental Figure S1A,B). Compared with the wild-type (WT) fruit, there were no significant differences in the *PbSWEET6*-OE fruit size and weight, but the SSC was significantly higher in the *PbSWEET6*-OE fruit (Figure 5A–C). Additionally, the fructose, glucose, and sucrose contents were significantly higher in *PbSWEET6*-OE fruit than in WT fruit (Figure 5D–F).

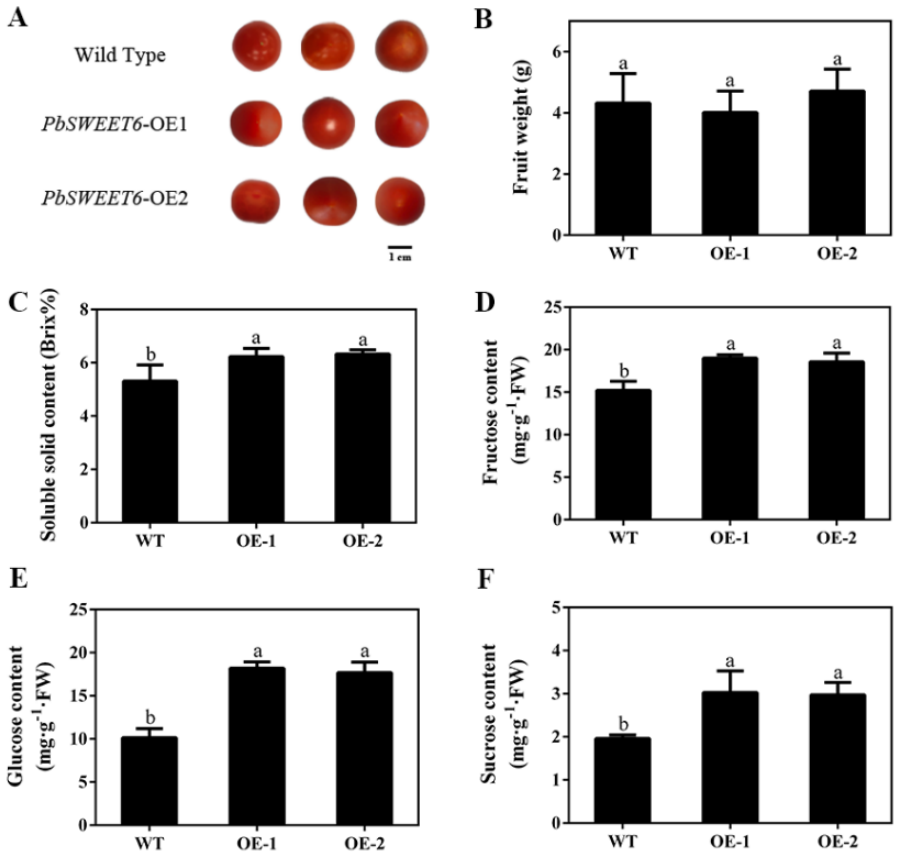

Figure 5. Fruit quality determination in wild type and *PbSWEET6*-OE tomato. (**A**) Fruits of wild type and *PbSWEET6*-OE lines at the red mature fruit stage; the scale bars correspond to 1 cm. (**B**) Single fruit weight. (**C**–**F**) The SSC and soluble sugar (fructose, glucose, and sucrose) contents in red mature fruits of the WT and *PbSWEET6*-OE lines. The data represent the mean values ± SDs. Different letters represent significant differences (Tukey's HSD, $p < 0.05$).

The *PbSWEET6* gene was also overexpressed in pear fruit calli, with an expression level that was significantly higher than that in the WT calli (Figure 6A,B). Furthermore, the glucose and sucrose contents were significantly higher in the *PbSWEET6*-OE fruit calli than in the WT fruit calli, whereas there were no significant differences in the fructose and sorbitol contents (Figure 6C–F).

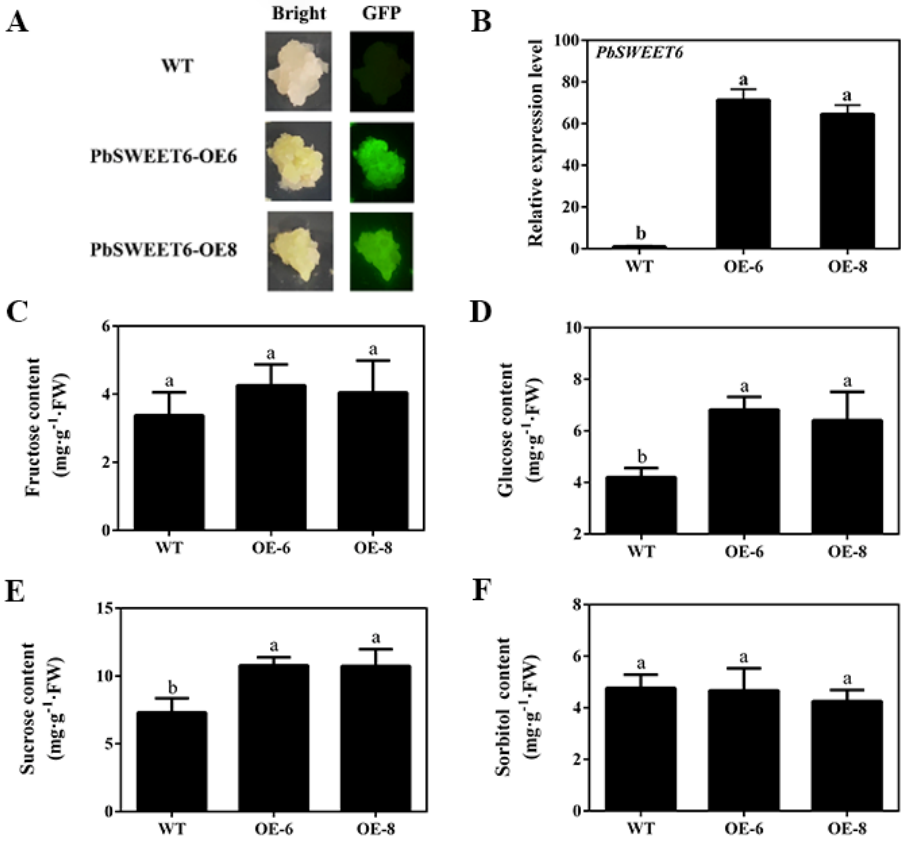

Figure 6. Effects of overexpressing *PbSWEET6* on sugar concentrations in pear fruit calli. (**A**) The transgenic pear fruit calli could be detected to express green fluorescent protein (GFP). OE-6 and OE-8 indicate the overexpressing *PbSWEET6* of pear fruit calli lines. (**B**) The qRT-PCR analysis of *PbSWEET6* expression levels in WT, OE-6 and OE-8 lines. (**C**–**F**) The soluble sugar (fructose, glucose, sucrose, and sorbitol) contents of WT, OE-6 and OE-8 pear fruit calli. The data represent the mean values ± SDs. Different letters represent significant differences (Tukey's HSD, $p < 0.05$).

4. Discussion

The rootstock significantly affects the vegetative and reproductive growth of the scion [32–35]. Quince is commonly used as a dwarfing rootstock for pear [36], resulting in precocious scion fruit development and increased fruit quality [4]. However, the molecular mechanism underlying the effects of quince rootstock on scion fruit quality remains largely unknown. In this study, 'Yunnan' quince significantly increased the weight and SSC of 'Zaosu' pear fruit. Moreover, the fructose, glucose, and sucrose contents were significantly greater in Z/Q fruit than in Z/D fruit. These results indicate that 'Yunnan' quince may be a better rootstock than 'Duli' for improving scion fruit quality.

Plants convert carbon dioxide and water into carbohydrates and oxygen through photosynthesis [37], which provides the energy and carbon necessary for plant growth and fruit formation. In tomato, the photosynthetic activity of green fruit influences the quality of ripe fruit [38]. Thus, photosynthesis has crucial effects on fruit quality. An examination of the leaves of 'Zaosu' pear grafted on 'Yunnan' quince and 'Duli' indicated there were no significant differences in the photosynthetic rate and chlorophyll concentration between the two combinations. Accordingly, the difference in the scion fruit sugar contents is likely unrelated to photosynthesis. In plants, photosynthates are transported from source organs to sink organs. Dwarfing rootstocks reportedly affect scion photosynthate transport. A previous study indicated that in apple, more photosynthates are transported to fruits when the scion is grafted on M9 and SH40 dwarfing rootstocks than when the scion is grafted on BC standard rootstocks [39]. Thus, we speculated that 'Yunnan' quince may facilitate the transport of photoassimilates to fruit. To assess this possibility, we performed ^{13}C

feeding experiments, which showed that 'Yunnan' quince promoted the distribution of photoassimilates to the fruit better than 'Duli', which was consistent with the results of prior research. Photoassimilates are transported from the source to the sink primarily as sugars. Hence, sugar distribution is a major determinant of fruit quality [40]. Therefore, the increase in the sugar content of 'Zaosu' pear fruit produced after grafting on 'Yunnan' quince may be associated mainly with changes in sugar transport.

Sucrose is one of the main forms of long-distance transport of assimilates. In plants, SWEET proteins can transport various sugars, including sucrose and fructose [14,26,41]. Of the four PbSWEET genes that were more highly expressed in Z/Q fruit than in Z/D fruit, *PbSWEET8* (Clade II of the *SWEET* family) as well as *PbSWEET10* and *PbSWEET14* (Clade I) likely encode hexose transporters, whereas *PbSWEET6* (Clade III) is probably involved in the transport of sucrose [9,14]. Among these four genes, the largest expression-level difference between the analyzed fruits was observed for PbSWEET6. Accordingly, it was selected for the follow-up study. Tissue-specific expression assays showed that *PbSWEET6* was highly expressed in the leaves, fruits, and carpopodium, which are involved in the transport of assimilates to fruit. These findings are in accordance with the expected sugar-transport-related function of *PbSWEET6*. Additionally, PbSWEET6 was localized in the plasma membrane, similar to PuSWEET15 and SlSWEET1a [13,26]. The phylogenetic analysis indicated that PbSWEET6 is most closely related to PuSWEET15 and AtSWEET15 (i.e., Clade III SWEETs). To further verify the *PbSWEET6* function, we overexpressed *PbSWEET6* in tomato plants and pear fruit calli. The *PbSWEET6*-OE tomato plants were shorter than the WT plants, possibly because of the increased transport of photoassimilates to fruit. An examination of sugar contents identified significant increases in the glucose and sucrose levels in tomato fruits and pear fruit calli. In contrast, the fructose content increased only in transgenic tomato fruits, which may reflect the differences in specific characteristics between tomato and pear (e.g., pear calli may have a lower glucose metabolism level). Although Clade III SWEET genes reportedly encode sucrose transporters, the clade also includes transporters of other sugars. For example, *SlSWEET14*, which belongs to Clade III, contributes to the transport of fructose, glucose, and sucrose [23]. In addition to transporting sucrose, *PbSWEET6* may also be involved in the transport of glucose. Therefore, high *PbSWEET6* expression levels might promote glucose and sucrose transport to increase the abundance of these sugars in fruit. Our findings may be useful for further clarifying how dwarfing rootstocks affect the transport of sugars into pear fruit.

5. Conclusions

'Yunnan' quince promotes the flow of photoassimilates to 'Zaosu' pear fruit, thereby increasing the fruit sugar content. The upregulated expression of *PbSWEET6* may be directly or indirectly involved in the accumulation of glucose and sucrose in 'Zaosu' pear fruit. Furthermore, overexpressed *PbSWEET6* influences the sucrose and glucose accumulation in tomato fruits and pear fruit calli. This study has expanded our understanding of how the regulation of *PbSWEET6* expression affects the fruit sugar content. This information may help breeders select pear rootstocks that can optimize fruit sugar contents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8070649/s1, Table S1: Primers used for relative expression analysis, gene cloning and vector construction; Figure S1: The detection and plant phenotype of transgenic tomato (OE-1, OE-2) overexpressing PbSWEET6.

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