SnRK1 phosphorylation of SDH positively regulates sorbitol metabolism and promotes sugar accumulation in peach fruit

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Fruit quality depends largely on the type and amount of sugar accumulated in the fruit. In peach [Prunus persica (L.) Batsch], sorbitol is the main photosynthetic product and plays a crucial role in sugar metabolism. As a conserved energy sensor, SNF1-related kinase 1 (SnRK1) is involved in the regulation of carbon metabolism. In this study, SnRK1 was able to respond to induction by treatment with exogenous trehalose and sorbitol on ‘Ruipan 17’ peach fruit. After treatment with 100-mM trehalose for 3 h, the SnRK1 activity decreased by 18% and the activities of sorbitol dehydrogenase (SDH) and sucrose synthase (SS) also decreased significantly, but sucrose phosphate synthase (SPS) activity increased significantly; whereas sorbitol treatment under the same conditions resulted in a 12.6% increase in SnRK1 activity and the activities of SDH and SS synthase also increased significantly, compared with the control. The contents of glucose, fructose and sucrose in peach fruit increased significantly after 3 h of sorbitol treatment. In addition, the interactions between PpSnRK1α and enzymes PpSDH and PpSPS were confirmed by yeast two-hybrid method and the phosphorylation of PpSnRK1α and PpSDH was detected in vitro. Taken together, these results suggest that SnRK1 promotes sorbitol metabolism by activating SDH and it also regulates the activities of SS and SPS that enhance sucrose accumulation in peach fruit. SnRK1 protein kinase is involved in sugar metabolism and has the potential to be used for improving fruit quality.

Keywords: fruit quality, Prunus persica, SDH activity, SnRK1 protein kinase, sorbitol, sugar metabolism.

Introduction

Sugar is the basic material for plant life activities, including fruit growth and development. Sugar components and contents have important effects on fruit internal quality formation and are the key parameters of determining fruit quality. In higher plants, sugar is not only an energy source and a structural component but also a powerful signal molecule capable of being sensed by plant cells (Lam et al. 1994, Jang et al. 1997). Sugar, hormone and nitrogen signaling constitutes a network that, through complex signal transduction mechanisms, regulates plant growth and gene expression (Rolland et al. 2002, León and Sheen 2003).

The sucrose non-fermenting-1 (SNF1)-related protein kinases are an important kinase family involved in sugar signaling and are emerging as major energy sensors in the metabolic signaling network controlling plant carbohydrate metabolism, growth and development, as well as stress tolerance (Halford and Hardie 1998, Polge and Thomas 2007, Lastdrager et al. 2014, Tsai and Gazzarrini 2014, Emanuelle et al. 2016, Hulsmans et al. 2016). SnRK1 kinase typically functions as a heterotrimeric complex that is composed of a catalytic subunit α (the actual kinase) and two regulatory subunits, β and γ, within plants (Ghillebert et al. 2011, Ramon et al. 2013, Emanuelle et al. 2016).

Many studies have shown that plant SnRK1 is involved in many metabolic pathways of plant life activities, not only carbohydrate metabolism but also seed filling, plant flowering,
maturation and senescence pathways (Purcell et al. 1998, Laurie et al. 2003, Baena-González et al. 2007, Jossier et al. 2009, Radchuk et al. 2010, Broeckx et al. 2016). Overexpression of \( \text{IbSnRK1} \) in sweet potato enhanced carbon assimilation (Ren et al. 2017). McKibbin et al. (2006) showed that sucrose synthase (SS) and ADP glucose pyrophosphorylase activity increased after over-expression of \( \text{SnRK1} \) in potato. In plants, \( \text{SnRK1} \) can regulate carbohydrate metabolism not only by directly regulating the expression of the \( \text{SS} \)-encoding gene at the transcriptional level (Purcell et al. 1998), but also by regulating some important metabolic enzyme activities through phosphorylation and dephosphorylation (Luo et al. 2020). These include 3-hydroxy-3-methylglutaryl-coenzyme A reductase, sucrose phosphate synthase (SPS) and \( \alpha \)-amylase, which have been shown to play important roles in carbon and amino acid signaling pathways (Sugden et al. 1999, Laurie et al. 2003, Halford et al. 2004, Hey et al. 2006, Baena-González et al. 2007, Radchuk et al. 2010). Schliepmann et al. (2003) found that with trehalose-6-phosphate (T6P) accumulation, the growth of wild-type Arabidopsis was inhibited in media with a high concentration of trehalose (100 mmol l\(^{-1} \)). Zhang et al. (2009) through in vitro experiments found that T6P inhibited \( \text{SnRK1} \) activity, and the inhibition of \( \text{SnRK1} \) activity was indirectly demonstrated by study of the expression of the \( \text{KIN10} \) (SNF1 kinase homolog 10) target genes.

In Rosaceae plants, sorbitol metabolism plays an important role in carbohydrate metabolism. Long-term studies showed that sorbitol was the major photosynthetic product of carbon transport and the main soluble storage carbohydrate in non-photosynthetic cells (Grant and Rees 1981, Loescher 1982, Priestley 1983). This effect of sorbitol on carbohydrate metabolism is characteristic of Rosaceae, and the role sorbitol played is like sucrose in other plants (Chong and Taper 1972). In the sink, sorbitol is mainly converted to fructose by sorbitol dehydrogenase (SDH) before entering the central metabolism (Negm and Loescher 1979, Oura et al. 2000).

The current study on the effect of \( \text{SnRK1} \) on sugar metabolism mainly focuses on Arabidopsis and some crops; however, in the Rosaceae fruit trees, the role of \( \text{SnRK1} \) in sugar metabolism pathways has been rarely reported. Our previous study showed that overexpression of \( \text{Malus hupehensis} \) \( \text{SnRK1} \). \( \text{SnRK1} \) in tomato increased the contents of starch and soluble sugar in fruits compared with wild-type tomato, and \( \text{MhSnRK1} \) had obvious effects on the activities of \( \text{SS}, \text{SPS} \) and \( \text{ADP-glucose pyrophosphorylase} \) in transgenic tomato leaves (Li et al. 2010, Wang et al. 2012). Overexpression of \( \text{PpSnRK1} \) \( \alpha \) subunit (\( \text{PpSnRK1}\alpha \)) in tomato also increased the content of soluble sugar in fruits and promoted fruit ripening compared with wild-type tomato (Yu et al. 2018). In this study, we investigated the response of \( \text{PpSnRK1} \) kinase to exogenous sugar signals as well as its effect on sorbitol and sucrose metabolism in peach fruit. We aimed to provide the theoretical basis for clarifying the regulation by \( \text{SnRK1} \) of fruit sugar metabolism and for improving fruit quality.

**Materials and methods**

**Materials and treatments**

This study was conducted at Hongmiao peach base in Shandong Taian, China, from 2015 to 2018. The fruit of 4-year-old ‘Ruipan 17’ peach trees were used as test material. At 35 days after full bloom, healthy fruits with uniform color and size were selected and randomly divided into three groups, with each group comprising 60 fruits. The first group of fruits was treated with 100 mmol l\(^{-1} \) trehalose solution by spraying. The treatment was with 100 mmol l\(^{-1} \) trehalose solution on one side and with distilled water on the other side, and the side of the distilled water was used as control. The second group of fruits was treated with 100 mmol l\(^{-1} \) sorbitol solution using the same method as for trehalose. And the last group of fruits was treated with 100 mmol l\(^{-1} \) sorbitol and trehalose mixed solution using the same method. Samples were respectively taken at 0, 1, 3 and 6 h after treatment. The flesh of 5-mm thickness on each side of the fruit surface was cut and stored in a freezer at \(-80{\text{ °C}}\) for the later determination of \( \text{SnRK1} \) activity, its subunit gene expression levels and enzyme activities related to sugar metabolism.

**\( \text{SnRK1} \) activity determination**

Fruit tissue (1 g) was ground in 1 ml of cold extraction buffer: 100 mmol l\(^{-1} \) HEPES, pH 8, 25 mmol l\(^{-1} \) NaF, 2 mmol l\(^{-1} \) sodium pyrophosphate, 0.5 mmol l\(^{-1} \) ethylene diamine tetra acetic acid, 0.5 mmol l\(^{-1} \) ethylene glycol tetra acetic acid, 1 mmol l\(^{-1} \) anisole, 5 mmol l\(^{-1} \) dithiothreitol, 25 mmol l\(^{-1} \) \( \beta \)-mercaptoethanol and 1 mol l\(^{-1} \) pepstatin A. The suspension was transferred to two cold microfuge tubes and clarified by centrifugation for 5 min at 12000 \( \times \) g at 4 °C. The supernatant (750 μl) was desalted on a 2.5-ml centrifuge column (Sephadex G-25 medium columns; GE Healthcare, Chalfont St Giles, UK) treated with equilibration solution. Using AMARA polypeptide as the substrate (Zhang et al. 2009, Debast et al. 2011), the \( \text{SnRK1} \) activity was measured using a Universal Kinase Activity Kit (R & D Systems, Minneapolis, MN, USA).

**Trehalose-6-phosphate determination**

Trehalose-6-phosphate (T6P) was extracted from 100 mg of peach fruit tissue with 500 μl of chloroform and 800 μl of methanol:water (5:3). Materials were then determined and analyzed using the method described by Debast et al. (2011).

**Determination of other enzyme activities in peach fruit**

Enzyme extraction methods refer to the method of Qi et al. (2006). The frozen sample (5 g) was ground in a small amount of quartz sand and 10 ml of HEPES buffer (50 mmol l\(^{-1} \)
HEPES-NaOH, pH 7.5, 1 mmol l\(^{-1}\) EDTA, 10 mmol l\(^{-1}\) MgCl\(_2\), 2.5 mmol l\(^{-1}\) DTT, 10 mmol l\(^{-1}\) ascorbic acid and 5% insoluble cross-linking polyvinylpyrrolidone), homogenized in an ice bath, filtered through four layers of gauze and centrifuged at 12,000 g (4 °C) for 20 min. Ammonium sulfate was gradually added to the supernatant at 80% saturation, 12,000 g for 20 min and the supernatant discarded. The precipitate was dissolved with 3 ml of extraction buffer and dialyzed for 20 h with 10-fold dilution of the extraction buffer (without cross-linking polyvinylpyrrolidone). All of the above operations were carried out at 0–4 °C. The SS catalyzed a reversible reaction, such that both SS synthesis activity and SS breakdown activity were measured. The SS activity was measured as described by Wang et al. (2012). The SPS activity was assayed in accordance with the procedure of Vassey (1989). All biochemical reagents, such as uridine diphosphate glucose and fructose-6-phosphate, were purchased from Sigma–Aldrich (St. Louis, MO, USA). The SDH activity was determined by a SDH kit purchased from Suzhou Keming Biotechnology Co. (Suzhou, China). Sorbitol oxidase (SOX) activity was determined by an SOX kit purchased from Suzhou Keming Biotechnology Co. Excel 2007 was used for data processing and IBM SPSS 22 software for statistical analysis.

**Determination of sorbitol, glucose, fructose and sucrose content in peach fruit**

The fruit sample (1 g) was ground completely using a mortar and pestle and placed in 20 ml of 80% ethanol at 70 °C for 10 min. The suspension was clarified by centrifugation for 10 min at 12,000 g. Supernatant was filtered through a 0.45-μm filter membrane and measured using the Beckman P/ACE 5000 high-performance capillary electrophoresis (Fullerton, CA, USA) by the method of Liu (2004).

**Construction of recombinant yeast two-hybrid expression vector and detection of protein interaction**

The amplification primers were designed according to the sequence of the enzyme-encoding genes on NCBI (http://www.ncbi.nlm.nih.gov/), and according to Clontech's pGAD424 and pGBT9 vector map. The pGAD-PpSnRK1α, pGBT-PpSDH, pGBT-PpSS and pGBT-PpSPS vectors were constructed and respectively transformed into Escherichia coli strain DH5α (TIANGEN, Beijing, China), and plasmids extracted for use. The AD-PpSnRK1α plasmids were co-transformed into the yeast strain Y2HGold (Clontech, Palo Alto, CA, USA) with BT-PpSDH, BT-PpSS and BT-PpSPS plasmids respectively, using the PEG/LiAC method as described in the Clontech Yeast Protocol Handbook. The colonies from the double selection plates (synthetic drop-out medium lacking leucine and tryptophan) were then screened for growth on quadruple selection SD medium lacking adenine, histidine, leucine and tryptophan (SD/Ade/His/Leu/Trp). To further confirm the positive interactions, X-α-Gal was used to assay for beta-galactosidase activity.

**In vitro kinase assays**

In vitro kinase assays were implemented according to the method described by Halfter et al. (2000). For in vitro kinase assays, the *E. coli* strain BL21-induced HIS-PpSnRK1α and HIS-PpSDH fused proteins were purified and co-incubated in kinase buffer (200 mM Tris–HCl, pH 7.2, 20 mM DTT, 50 mM MgCl\(_2\), 100 μM ATP and 50 mM CaCl\(_2\)) containing 10 μCi of [γ\(^{-32}\)P] ATP at 30 °C for 30 min. Proteins were separated by SDS–PAGE, and phosphorylated protein was detected by autoradiography.

**Determination of PpSDH activity after PpSnRK1α reacts with PpSDH**

The ELISA method was used to determine PpSDH activity after PpSnRK1α reacted with PpSDH. Purified HIS-PpSnRK1α and HIS-PpSDH fused proteins were co-incubated in kinase buffer (200 mM Tris–HCl, pH 7.2, 20 mM DTT, 50 mM MgCl\(_2\), 100 μM ATP and 50 mM CaCl\(_2\)) at 30 °C for 30 min. Then the SDH activity was measured with Plant SDH ELISA Kit (MB-10747B, Jiangsu Meibiao Biological Technology, Yancheng, China). Enzyme activity was expressed in U l\(^{-1}\).

**Statistical analysis**

Three independent biological replicates were performed for each experiment. Microsoft Office Excel 2007 was used for data processing and IBM SPSS 22 software for statistical analysis. Significance was defined as *P* < 0.05.

**Results**

**Effects of exogenous trehalose and sorbitol on SnRK1 activity in peach fruit**

SnRK1 was able to respond to induction by treatment with exogenous trehalose and sorbitol on ‘Ruian 17’ peach fruit. After treatment with exogenous trehalose for 1 h, the content of T6P increased significantly in peach fruit (Figure 1A). Trehalose-6-phosphate (T6P) inhibited SnRK1 activity (Zhang et al. 2009). The activity of SnRK1 decreased significantly after treatment with trehalose, decreasing by 18% after treatment for 3 h (Figure 1B). And the SnRK1 activity increased SnRK1 activity in peach fruit, increasing it by 12.6% for 3 h (Figure 1B). In contrast, exogenous sorbitol treatment decreased after treatment with trehalose and sorbitol co-treatment but the decrease is less than when treated with trehalose alone (Figure 1D).

**Effects of exogenous trehalose and sorbitol on activities of SDH, SOX, SPS and SS in peach fruit**

After treatment with trehalose and sorbitol, significant differences were observed in the activities of SDH, SOX, SPS and SS in peach fruits (Figure 2). After 3 h of treatment with 100-mM trehalose, the activities of SDH and SS synthase in peach fruit decreased significantly compared with the control.
Figure 1. Effect of trehalose and sorbitol treatments on SnRK1 activity of peach fruit. (A) The content of trehalose-6-phosphate in peach fruit after the treatment with 100-mM trehalose. An asterisk (*) on top of the error bar designates a significant difference compared with the control at \( P < 0.05 \). (B) SnRK1 activity under the trehalose treatment. (C) SnRK1 activity under the sorbitol treatment. (D) SnRK1 activity under the trehalose and sorbitol co-treatment. Error bars represent the SD based on three independent biological replicates. The various small letters indicate significant differences compared with the control separately at each sampling time at \( P < 0.05 \) level.

(Figure 2A and D), whereas the SPS activity increased significantly compared with the control (Figure 2C). The changes in SOX activity and SS decomposition activity were not compared with the control (Figure 2B and E).

After 1–3 h of 100-mM sorbitol treatment, the activities of SDH and SS synthase increased significantly, whereas SPS activity and SS decomposition activity decreased significantly, compared with the control (Figure 2A and C–E).

In the case of co-treatment with 100 mM sorbitol and trehalose, the changes in activities of SDH, SPS and SS were similar to those of treatment with trehalose alone, but the range of change was significantly reduced (Figure 2A and C–E). And the changes in activities of SDH, SPS and SS were always correlated with the changes of SnRK1 activity. It has been reported that SnRK1 kinase can regulate SPS and SS in sugar metabolism, and we speculate whether there is regulation between SnRK1 kinase and SDH.

**Effects of exogenous trehalose and sorbitol on contents of sorbitol, glucose, fructose and sucrose in peach fruit**

The contents of fructose and sucrose in peach fruit decreased significantly after 3 h of trehalose treatment (Figure 3B and D). Trehalose treatment increases sorbitol levels (Figure 3A), and this would be mediated by decreased SDH activity. With the exception of sorbitol, the contents of glucose, fructose and sucrose significantly increased in the sorbitol-treated fruit after 3 h (Figure 3B–D). The sorbitol treatment increased the sugar content of the fruit, while trehalose had some inhibitory effect on sugar accumulation.

**Interactions of SnRK1α with SDH, SS and SPS identified by yeast two-hybrid system**

The pGAD vectors containing PpSnRK1α were co-transfected into yeast with pGBT vectors, respectively, containing PpSDH-, PpSS- and PpSPS-encoding genes. On SD/−Trp/−Leu/−His/−Ade, the yeast with the PpSnRK1α–PpSS did not grow normally (Figure 4). However, PpSnRK1α–PpSDH- and PpSnRK1α–PpSPS-transformed yeast grew normally on SD/−Trp/−Leu/−His/−Ade and were stained blue by X-α-gal, indicating PpSnRK1α–PpSDH and PpSnRK1α–PpSPS interactions (Figure 4).

**PpSnRK1α phosphorylates PpSDH protein**

PpSnRK1 is a Ser/Thr protein kinase. To examine whether PpSnRK1α phosphorylates PpSDH protein, purified HIS-PpSnRK1α and HIS-PpSDH proteins were used for an in
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Figure 2. Effect of trehalose and sorbitol treatments on activities of SDH, SOX, SPS and SS in peach fruit. (A) SDH activity; (B) SOX activity; (C) SPS activity; (D) SS synthase activity; (E) SS decomposition activity. Error bars represent the SD based on three independent biological replicates. An asterisk (*) on top of the error bar designates a significant difference compared with the control separately at each sampling time at $P < 0.05$.

In vitro phosphorylation experiment (Figure 5A and B). The result showed that PpSnRK1α efficiently autophosphorylated itself, indicating its kinase activity. The phosphorylated PpSDH protein was detected in the PpSnRK1α and PpSDH co-incubated sample, but not in the PpSDH sample alone, indicating that PpSnRK1α directly phosphorylated PpSDH protein in vitro (Figure 5C).

**PpSnRK1α phosphorylation of PpSDH increases PpSDH activity**

In order to show whether the phosphorylation event actually impacts SDH activity, enzyme assays were performed using a purified protein system where we monitored SDH activity using PpSnRK1α phosphorylated PpSDH vs non-phosphorylated PpSDH. The results showed that the activity of PpSnRK1α phosphorylated PpSDH increased significantly compared with the unphosphorylated PpSDH activity (Figure 6).

**Discussion**

Sweetness is a major parameter of determining fruit organoleptic quality, and its intensity mainly depends on sugar composition and content (Kroger et al. 2006). In higher plants, sugar is not only a transport and storage form of photosynthate and energy, but also a powerful signal molecule capable of being sensed by plant cells, which in turn regulates gene expression and affects physiological and biochemical processes (Lam et al. 1994).

SnRK1 in plants is a crucial kinase family involved in sugar signaling and its activity can be regulated by sugar (Halford and Hardie 1998, Baena-González and Sheen 2008, Jossier et al. 2009). In this study, it was found that SnRK1 activity was regulated by exogenous trehalose and sorbitol, and the regulation of the two sugars was completely opposite. Trehalose is a non-reducing disaccharide, thought to be an osmolyte commonly found in plants (Wingler 2002, Voit 2003). The precursor for the synthesis of trehalose is T6P, which inhibits
Figure 3. Effect of trehalose and sorbitol treatments on contents of sorbitol, fructose, glucose and sucrose in peach fruit. (A) The content of sorbitol; (B) the content of fructose; (C) the content of glucose; (D) the content of sucrose. Error bars represent the SD based on three independent biological replicates. An asterisk (*) on top of the error bar designates a significant difference compared with the control separately at each sampling time at $P < 0.05$.

Figure 4. Yeast two-hybrid experiments showing the interactions between SnRK1 and the SDH, SS and SPS proteins. Results show a representative experiment out of three independent biological replicates.

SnRK1 activity in both wheat and potato tubers (Martíne-Barajas et al. 2011). Zhang et al. (2009) found that T6P content was high in the presence of sufficiently available carbon, and T6P inhibited SnRK1 activity. When available carbon is lacking, the T6P content decreases and SnRK1 activity increases to decrease the pathway of consumption of the carbon source and increases catabolism and the photosynthetic process (Baena-González et al. 2007, Baena-González and Sheen 2008). In our study, SnRK1 activity decreased in the short-term following treatment with 100 mM trehalose, decreasing by 18% after 3 h of treatment.

Peach (Prunus persica (L.) Batsch) belongs to the Rosaceae family and its main photosynthate is sorbitol (Chong and Taper 1972, Moing et al. 1997). In this study, it was found that treatment with 100-mM sorbitol on peach fruit after 3 h resulted in a 12.6% increase in SnRK1 activity. Chong and Taper (1972) mentioned that sorbitol has similar roles as sucrose in other species, but that probably only goes for its role as transport sugar. Sucrose inhibits SnRK1 activity Baena-González et al.
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Figure 5. Phosphorylation detection of PpSnRK1α and PpSDH in vitro. (A) The purified PpSnRK1α protein. MK: molecular weight marker; PpSnRK1α: PpSnRK1α protein. (B) The purified PpSDH protein. MK: molecular weight marker; PpSDH: PpSDH protein. (C) PpSnRK1α phosphorylates PpSDH in vitro. The asterisk indicates the autophosphorylation of the purified HIS-PpSnRK1α, while the triangle indicates the phosphorylation of the purified HIS-PpSDH by HIS-PpSnRK1α in the autoradiogram.

Figure 6. Effect of PpSnRK1α phosphorylation on PpSDH activity. Error bars represent the SD. Different small letters indicate significant differences at P < 0.05 level.
2007. Our research is the first to show that sorbitol increases SnRK1 activity; however, the exact mechanism of this remains unclear, pending further study. Sugar metabolism in peach fruit comprises a complex regulatory network and there are a variety of enzymes involved in it, such as fructokinase (FK), SDH, SS, SPS and amylase (Moriguchi et al. 1990, Rolland et al. 2006). SnRK1 is considered as an important carbon metabolic regulator, which has been proved to activate SS and α-amylase and inactivate SPS and trehalose phosphate synthase 5 (TPS5) to regulate nutrient balance (Sugden et al. 1999, Halford et al. 2003, Harthill et al. 2006, Polge and Thomas 2007). In this study, after treatment with trehalose, SnRK1 activity decreased, and activities of the key enzymes SDH and SS synthase of sugar metabolism also decreased as the SnRK1 activity decreased, whereas SPS activity increased. In addition, when SnRK1 activity in peach fruit increased after treatment with 100 mM sorbitol, the activities of SDH, SPS and SS synthase also changed with the change in SnRK1 activity. These results demonstrate the correlation between SnRK1 activity and the activities of SDH, SS synthase and SPS. Using yeast two-hybrid assay, we verified whether SnRK1 interacted with the three metabolic enzymes. The result showed that the PpSnRK1α interacted with PpSDH and PpSPS. Furthermore, the phosphorylation of PpSNRK1α and PpSDH was also detected in vitro. The PpSNRK1α-PpSDH interactions were confirmed in fruit trees for the first time.

There are various types of soluble sugars accumulated during peach fruit development, such as sorbitol, inositol, glucose, fructose and sucrose (Cantín et al. 2010, Monti et al. 2016). This study has indicated that SnRK1 activated SDH and SS synthase, which led to the increase in fructose and sucrose. Increased SnRK1 activity in fruits is also correlated with higher sucrose accumulation while SPS activity is inhibited. Moriguchi et al. (1992) studied 23 kinds of pears and found that the correlation coefficient between SS activity and sucrose content in fruits was 0.633, while the correlation coefficient between SPS activity and sucrose content was only 0.445. Studies on sugarcane and peach fruit also show that SS contributes more to sucrose accumulation than SPS. Our research is the first to show that sorbitol increases SnRK1 activity; however, the exact mechanism of this remains unclear, pending further study. In immature fruit, the major sugars are glucose and fructose, whereas in ripe fruit, sucrose acts as the predominant soluble sugar. Sweetness is a crucial component of fruit quality, and peach fruit sweetness mainly depends on sucrose content (Cirilli et al. 2016). In plants, SnRK1 can regulate carbohydrate metabolism not only by regulating some important metabolic enzyme activities through phosphorylation, but also by directly regulating the expression of the SS-encoding gene at the transcriptional level (Purcell et al. 1998). There may be another layer of regulation of SDH and SPS by SnRK1 in response to exogenous sorbitol beyond phosphorylation of SDH and SPS by SnRK1, i.e., transcriptional regulation of SDH and SS directly by SnRK1 or via an unknown transcription factor that is phosphorylated by SnRK1. However, these questions still need further research. For the first time, our findings reveal that SnRK1 activity is increased by sorbitol and SnRK1 can heighten sorbitol metabolism by activating the key enzyme SDH. SnRK1 also enhances sucrose accumulation by regulating the activities of SS and SPS in peach fruit. This study suggests that SnRK1 is involved in sorbitol metabolism and has the potential to be used for improving fruit quality.

Authors’ contributions
F.P. and W.Y. conceived and designed the experiments. W.Y., W.W. and J.L. performed the experiments. F.P., W.W., Y.X. and X.Y. contributed reagents, materials and analysis tools. W.Y. and F.P. wrote the paper.

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Conflict of interest
The authors declare no competing financial interest.

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