A sucrose non-fermenting-1-related protein kinase 1 gene from potato, *StSnRK1*, regulates carbohydrate metabolism in transgenic tobacco

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Abstract Sucrose non-fermenting-1-related protein kinase 1 (SnRK1) has been shown to play an essential role in regulating saccharide metabolism and starch biosynthesis of plant. The regulatory role of *StSnRK1* from potato in regulating carbohydrate metabolism and starch accumulation has not been investigated. In this work, a cDNA encoding the SnRK1 protein, named *StSnRK1*, was isolated from potato. The open reading frame contained 1545 nucleotides encoding 514 amino acids. Subcellular localization analysis in onion epidermal cells indicated that *StSnRK1* protein was localized to the nucleus. The coding region of *StSnRK1* was cloned into a binary vector under the control of 35S promoter and then transformed into tobacco to obtain transgenic plants. Transgenic tobacco plants expressing *StSnRK1* were shown to have a significant increased accumulation of starch content, as well as sucrose, glucose and fructose content. Real-time quantitative PCR analysis indicated that overexpression of *StSnRK1* up-regulated the expression of sucrose synthase (*NtSUS*), ADP-glucose pyrophosphorylase (*NtAGPase*) and soluble starch synthase (*NtSSS III*) genes involved in starch biosynthesis in the transgenic plants. In contrast, the

expression of sucrose phosphate synthase (*NtSPS*) gene was decreased in the transgenic plants. Meanwhile, enzymatic analyses indicated that the activities of major enzymes (SUS, AGPase and SSS) involved in the starch biosynthesis were enhanced, whereas SPS activity was decreased in the transgenic plants compared to the wild-type. These results suggest that the manipulation of *StSnRK1* expression might be used for improving quality of plants in the future.

Keywords Potato · Soluble sugar · Starch content · *StSnRK1* · Tobacco

Introduction

Biofuel significantly reduces the extraction and use of fossil fuels and therefore decrease environmental damage. There is a global awareness on breeding of non-food energy crops and research on biofuels is under progress (Sanz-Barrio et al. 2013; Wang et al. 2016a). Starch has been used as a main raw material for making first-generation biofuels due to the relatively easy conversion of starch into fermentable sugars (Smith 2008; Sanz-Barrio et al. 2013). Therefore, it is very important to understand how carbohydrates are metabolized in plants, which could greatly help to promote starch synthesis and improve the production efficiency of bio-fuels (Sanz-Barrio et al. 2013; Wang et al. 2016c).

There are three subfamilies of sucrose non-fermenting-1 (SNF1)-related protein kinases [SnRKs] (Halford and Hardie 1998; Celenza and Carlson 1986; Jossier et al. 2009) in higher plants viz. SnRK1, SnRK2 and SnRK3 (Hrabak et al. 2003). Carbohydrate metabolism and starch

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biosynthesis are regulated by SnRK1 in plants (Polge and Thomas 2007; Halford and Hey 2009; Wang et al. 2012).

Conversion of sucrose to starch is mediated by two key enzymes namely sucrose synthase (SUS) and ADP-glucose pyrophosphorylase (AGPase, Purcell et al. 1998; McKibbin et al. 2006; Jiang et al. 2013). The redox modulation of AGPase activity is necessary for the reaction of sucrose (Tiessen et al. 2003) and regulates the expression of these two genes associated with biosynthetic pathway.

In plants, *SnRK1* from the rye was the first such gene, which was cloned and characterized (Alderson et al. 1991). Subsequently, several *SnRK1* genes have been identified from different plant species such as *Arabidopsis* (Kleinow et al. 2000), maize (Lumbreras et al. 2001), apple (Li et al. 2010) and sweet potato (Jiang et al. 2013). There are a few reports about investigating the function of *SnRK1* on carbohydrate metabolism and starch biosynthesis in plants. The inhibition of *SnRK1* function by antisense approach showed reduced viability and almost complete loss of starch accumulation in developing pollen grains (Zhang et al. 2001). Kanegae et al. (2005) found that *SnRK1* was involved in starch accumulation in rice. The study of McKibbin et al. (2006) reported that overexpression of *SnRK1* increased *SUS* and *AGPase* expression and enhanced their enzyme activities, leading to increased starch levels in the potato tubers. Jain et al. (2008) further found that the expression of a *SnRK1* gene occurred simultaneously with the accumulation of starch in sorghum endosperm and maize endosperm. Wang et al. (2012) and Jiang et al. (2013) demonstrated that overexpression of *SnRK1* from apple/sweet potato increased soluble sugar and starch content by increasing the expression of *SUS* and *AGPase* and enhancing the activities of *SUS* and *AGPase* in transgenic tomato/tobacco plants. It is thought that SnRK1 transfers carbon to starch by means of the storage pathway (McKibbin et al. 2006; Halford and Hey 2009). Furthermore, SnRK1 can inactivate sucrose phosphate synthase (SPS) via phosphorylation to regulate carbohydrate metabolism (Sugden et al. 1999; Halford and Hey 2009). SPS activity has been shown to decrease in the transgenic tomato/tobacco plants overexpressing *SnRK1* from apple/sweet potato (Wang et al. 2012; Jiang et al. 2013).

There are information available for *SnRK1* characterization in different plant species but the regulatory role of *StSnRK1* (Genbank accession No. NP_001274953) from potato in regulating carbohydrate metabolism and starch accumulation has not been reported. In this work, we isolated *StSnRK1* from potato and elucidated its roles in transgenic tobacco. *StSnRK1*-overexpressing tobacco and wild type (WT) tobacco were used to investigate the function of *StSnRK1* on carbohydrate metabolism. Overexpression of *StSnRK1* was found to significantly increase

sucrose, glucose, fructose and starch content in the leaves of transgenic tobacco plants, suggesting a potential use of *StSnRK1* in improving quality levels of plants in the future.

Materials and methods

Plant materials

Potato cultivar Zhongshu No. 5 was employed for *StSnRK1* gene cloning in this study. Tobacco (*Nicotiana tabacum* L.) cultivar Wisconsin 38 [wild type (WT)], a model plant, was used to study the functions of *StSnRK1*.

Cloning and sequence analysis of the potato *StSnRK1* gene

Total RNA was extracted from the leaves of Zhongshu No. 5 with the RNA prep Pure Kit (Tiangen Biotech, Beijing, China). RNA samples were reverse-transcribed according to the instructions of Quant script Reverse Transcriptase Kit (Tiangen Biotech, Beijing, China). Based on the sequence of *StSnRK1* (Genbank accession No. NP_001274953), we designed one gene-specific primers (GC-F/R) of reverse transcription PCR (RT-PCR) (Table S1) to obtain its full-length cDNA sequence. PCR was performed with an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products were separated on a 1.0% (w/v) agarose gel. Target DNA band was recovered by gel extraction, then cloned into PMD19-T (TaKaRa, Beijing, China), and finally transformed into competent cells of *Escherichia coli* strain DH5 α . White colonies were checked by PCR and the positive colonies were sequenced (Invitrogen, Beijing, China).

The open reading frame (ORF) of the cloned *StSnRK1* gene was predicted with ORF Finder (<http://www.ncbi.nlm.nih.gov/projects/gorf/>). The theoretical molecular weight and isoelectric point (pI) of the StSnRK1 protein were calculated using <http://web.expasy.org/protparam/>. The nuclear localization signal of StSnRK1 protein was predicted using cNLS Mapper program (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi). The conserved domain of StSnRK1 protein was scanned by the InterProScan program (<http://www.ebi.ac.uk/Tools/pfa/iprscan/>). The homology of StSnRK1 protein was identified using protein BLAST in the National Center for Biotechnology Information (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A multiple sequence alignment of StSnRK1 with other SnRK1 proteins from different plant species retrieved from NCBI was conducted using the DNAMAN software (Lynnon Biosoft, Quebec, Canada).

Phylogenetic analysis was conducted using the MEGA4 software (<http://www.megasoftware.net/>).

Subcellular localization of StSnRK1

Subcellular localization of StSnRK1 in onion (*Allium cepa*) epidermal cells was analyzed as described by Wang et al. (2016b). The ORF of *StSnRK1* was cloned and then inserted into the pMDC83 expressing vector containing the green fluorescent protein gene (GFP) at *SpeI* and *AscI* restriction sites under the control of the CaMV35S promoter and NOS (nopaline synthase) terminator (Table S1). Both the fusion construct (StSnRK1-GFP) and the control vector (GFP) were transformed into living onion epidermal cells by particle bombardment with a GeneGun (Biorad HeliosTM) according to the instruction manual (helium pressure 260 psi). After incubation on MS medium (pH 5.8) solidified with 3% agar at 28 °C for 24–36 h, the onion cells were observed with bright field and fluorescence using confocal microscopy (Nikon Inc., Melville, NY).

Transformation of tobacco with *StSnRK1*

The binary vector pCambia1301-*StSnRK1* used in this study contained the *StSnRK1* gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) terminator and β -glucuronidase (*gusA*) and hygromycin resistance (*hptII*) genes driven by a CaMV 35S promoter, respectively. The primers used to amplify *StSnRK1* were designed with the Primer5 (Table S1). The vector Cambia1301-*StSnRK1* was transformed into the *Agrobacterium tumefaciens* strain EHA105 cells by the electroporation method for tobacco transformation (Lou et al. 2007). Transformation and plant regeneration were performed according to the method of Jiang et al. (2013).

Molecular confirmation of transgenic plants

The putatively transgenic tobacco plants were identified using the histochemical GUS assay according to Jefferson et al. (1987). The blue staining of tissues indicated a positive reaction. Genomic DNA was extracted from the leaves of the GUS-positive plants, and PCR amplifications were performed using specific primers (Table S1) to amplify fragments of the *hptII* coding sequence.

Analyses of sucrose, glucose, fructose and starch content

The content of sucrose, glucose and fructose were measured using the anthrone method according to the method

of Zhang (1977). Starch extraction and quantification were performed based on the means of Smith and Zeeman (2006). The transgenic plants and WT were grown in pots containing a mixture of soil, vermiculite and humus (1:1:1, v/v/v) under normal conditions for 4 weeks at 27 °C under standard long day conditions (14 h light and 10 h dark). Leaves of plants were harvested to determine sucrose, glucose, fructose and starch content in light at 10–11 a.m. All treatments were performed in triplicate.

Southern blot analysis

Genomic DNA was extracted from the leaves of transgenic and WT plants by cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1985). Southern blot analysis was conducted as described by Wang et al. (2016c). Coding sequence of the 501bp *StSnRK1* was used as probe (Table S1). The labeling of probe, prehybridization, hybridization and detection were performed using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics GmbH, Germany).

Expression analysis of the related genes

The expression of *StSnRK1* and starch biosynthesis related genes was analyzed by real-time quantitative PCR (qRT-PCR) as described by Wang et al. (2016c). The cDNA solution was used as templates for PCR amplification with gene specific primers (Table S1). Tobacco *Nt actin* gene was used as an internal control (Jiang et al. 2013) (Table S1). Quantification of gene expression was done with comparative C_T method (Schmittgen and Livak 2008). All experiments were repeated three times and each data represents the average of three experiments.

SnRK1, SUS, SPS, AGPase and SSS activity assays

The activities of SnRK1, SUS, SPS, AGPase and SSS involved in starch biosynthesis were measured using the methods described by Nakamura et al. (1989), Zhang et al. (2009), McKibbin et al. (2006) and Wang et al. (2012).

Statistical analysis

All experiments were repeated three times and the data presented as the mean $\pm$ standard error (SE). Where applicable, data were analyzed by Student's *t* test in a two-tailed analysis. Values of $P < 0.05$ or < 0.01 was considered to be statistically significant difference.

Results

Cloning and sequence analysis of *StSnRK1*

The *StSnRK1* gene contained a 1545 bp ORF encoding a 514 amino acids polypeptide with a molecular weight of 58.74 kDa and a *pI* of 8.03. The InterProScan domain analyses of *StSnRK1* amino acid sequence showed the presence of Serine/Threonine-protein kinase catalytic domain (S-TKc) and the Serine/Threonine-protein kinase active site at 138–150 position, which are the characteristic features of plant SnRK1 protein (Fig. 1a). The cNLS mapper program detected a putative nuclear localization signal (NLS) at 222–233 position in the sequence (Fig. 1a).

A BLAST search showed that the amino acid sequence of *StSnRK1* showed a high amino acid identity with predicted protein products of *Solanum lycopersicum* (NP_001304105, 97.67%), *Nicotiana tomentosiformis* (XP_009621306, 94.75%), *Malus domestica* (XP_008365408, 86.02%), *Arachis duranensis* (XP_015946108, 85.05%), *Theobroma cacao* (XP_007032620, 84.82%), *Vigna radiate* (XP_014512885, 84.11%), *Cajanus cajan* (KYP60405, 83.33%), *Cice rarietinum* (XP_004489425, 83.11%), *Arabidopsis thaliana* (NP_566130, 82.72%) *Glycine max* (NM_001251194, 82.17%) and *Solanum tuberosum* (CAA65244, 65.83%) (Fig. 1a). Phylogenetic analysis showed that *StSnRK1* is closely related with predicted SnRK protein products of *S. lycopersicum* (Fig. 2).

Nuclear localization of *StSnRK1*

The subcellular localization of *StSnRK1* protein was investigated in onion epidermal cells. During transient expression experiment, we observed the fluorescence of *StSnRK1*-GFP exclusively localized in the nuclei of the cells (Fig. 3). The control experimental set showed that GFP fluorescence was present throughout cells (Fig. 3). These results suggests that *StSnRK1* is a nuclear-localized protein and therefore may be involved in the regulation of downstream gene targets.

Regeneration and identification of transgenic tobacco with *StSnRK1* gene

The binary vector containing *StSnRK1* ORF (pCAM-BIA1301-*StSnRK1*) was used to ectopically express the gene in tobacco (Fig. 4a). A total of 250 leaf discs of tobacco cultivar Wisconsin 38 co-cultivated with the *A. tumefaciens* produced 24 putatively transgenic plants from 24 leaf discs. GUS assay showed that 6 of them had visible GUS activity in leaf, stem and root tissues, indicating stable integration of *gusA* gene in the genome of the

plants (Fig. 4b, c, d). We could not detect the GUS expression in the remaining 18 transgenic and WT plants (Fig. 4b, c, d). Six independent transgenic lines overexpressing *StSnRK1* were obtained by Hyg resistance selection, numbered #1–#6, respectively. PCR analysis further confirmed that these 6 plants were transgenic (Fig. 4e).

Assay of sucrose, glucose, fructose and starch content in transgenic tobacco

Quantitative transcript analyses by qPCR showed the significantly increased expression level of *StSnRK1* in the transgenic lines, especially #2, #3 and #4, while no transgene expression was observed in WT (Fig. 5). Furthermore, we found that SnRK1 activity was significantly enhanced in transgenic lines (#2, #3 and #4) (Fig. 6). Therefore, transgenic lines #2, #3 and #4 were selected for further analysis.

The transgenic plants (lines #2, #3 and #4) and WT, grown in pots under normal condition for 4 weeks, had no significant differences in growth (Fig. S1). However, the sucrose, glucose, fructose and starch content in the leaves of these plants were different. The results showed that the *StSnRK1*-over expressing tobacco plants had significantly higher sucrose, glucose, fructose, soluble sugar and starch content, which were increased by 34–92%, 62–234%, 19–31%, 42–54% and 14–34%, respectively, compared to WT (Figs. 7, 8).

Southern blot analysis of transgenic tobacco plants

Southern blot analysis indicated that 3 transgenic plants (lines #2, #3 and #4) with higher starch content displayed different integration patterns. The copy number of integrated *StSnRK1* gene varied from 1 to 2. We could not detect any evident relationship between expression levels of related genes and copy number of integrated *StSnRK1* gene (Fig. 9). Also, no clear relationship between the starch accumulation and the copy number was ascertained (Fig. 9).

Expression analysis of starch biosynthetic genes in transgenic tobacco plants

qRT-PCR analysis showed that sucrose synthase (*NtSUS*), AGPase (*NtAGPase*) and soluble starch synthase (*NtSSS III*) genes related to starch biosynthesis pathway were all up-regulated (Fig. 10), whereas the expression level of *NtSPS* was decreased in transgenic plants (Fig. 10). These results demonstrated that *StSnRK1* gene might be involved in the regulation of starch biosynthetic processes.

(XP_015946108), *Cajanuscajan* (KYP60405), *Cicerarietinum* (XP_004489425), *Glycine max* (NM_001251194), *Malusdomestica* (XP_008365408), *Nicotianatomentosiformis* (XP_009621306), *Solanumlycopersicum* (NP_001304105), *Solanumtuberosum* (CAA65244), *Theobroma cacao* (XP_007032620) and *Vignaradiata* (XP_014512885)

Fig. 2 Phylogenetic tree of the StSnRK1 protein with its homologous proteins from other plant species. The proteins are as follows: *Arabidopsis thaliana* (NP_566130), *Arachis duranensis* (XP_015946108), *Cajanus cajan* (KYP60405), *Cicer arietinum* (XP_004489425), *Glycine max* (NM_001251194), *Malus domestica* (XP_008365408), *Nicotiana tomentosiformis* (XP_009621306), *Solanum lycopersicum* (NP_001304105), *Solanum tuberosum* (CAA65244), *Theobroma cacao* (XP_007032620) and *Vigna radiata* (XP_014512885). The branch lengths are proportional to distance

Enzyme activity assays in transgenic tobacco plants

The enzymatic analyses indicated that the activities of SUS, AGPase and SSS were significantly enhanced, whereas the activity of SPS was decreased in transgenic plants compared to that in the WT (Fig. 11). The changes in enzyme activity of transgenic plants showed correlation with the transcription levels of their corresponding genes. All these findings indicate that *StSnRK1* has significant effects on the activities of SUS, SPS, AGPase and SSS in transgenic tobacco plants.

Fig. 3 Subcellular localization of the StSnRK1 protein in onion epidermal cells. The StSnRK1-GFP fusion protein was localized to the nucleus. Scale bar = 100 μ m

Discussion

There are a few findings that SnRK1 is involved in carbohydrate metabolism and starch biosynthesis of plants. Kanegae et al. (2005) reported that *SnRK1* had a role in starch accumulation in rice. The inhibition of *SnRK1* led to the almost complete loss of starch accumulation and viability in developing pollen grains (Zhang et al. 2001). Overexpression of *SnRK1* from apple/sweet potato increased soluble sugar and starch content in transgenic tomato/tobacco plants (Wang et al. 2012; Jiang et al. 2013). In this work, we isolated a *StSnRK1* gene from potato. Bioinformatic analyses of StSnRK1 showed the presence of characteristic domain present in plant SnRK1 proteins (Fig. 1). Transient expression of StSnRK1 in onion epidermal cells suggest StSnRK1 protein is localized to the nucleus (Fig. 3), similar to the results of Vincent et al. (2001) and Jiang et al. (2013). Our results also revealed that overexpression of *StSnRK1* was found to increase soluble sugar and starch content in transgenic tobacco plants (Figs. 7, 8).

The reversible conversion of sucrose and UDP to UDP-glucose (UDPG) and fructose is catalyzed by enzyme SUS (Lee et al. 2009, 2010; Ovono et al. 2009; Jiang et al. 2013). The SUS activity was closely related to the strength of the sink and the accumulation of starch (Zrenner et al. 1995; Jiang et al. 2013; Wang et al. 2016a). SPS is involved in the conversion of UDPG and fructose into sucrose (Sugden et al. 1999; Halford and Hey 2009). AGPase converts G-1-P to ADP-glucose (ADPG) (Tiessen et al. 2003; Geigenberger 2011). It is reported that AGPase is regulated by transcriptional redox, which provides a mechanism for regulating the starch synthesis rate in potato

Fig. 4 Molecular confirmation of transgenic plants. **a** Schematic diagram of the T-DNA region of binary plasmid pCAMBIA1301-*StSnRK1*. LB, left border; RB, right border; *hptII*, hygromycin phosphotransferase II gene; *StSnRK1*, potato sucrose non-fermenting-1 related protein kinase 1 gene; *gusA*, β -glucuronidase gene; 35S, cauliflower mosaic virus (CaMV) 35S promoter; 35S T, CaMV 35S terminator; NOS T, nopaline synthase terminator. **b**, **c** and **d** GUS

expression in leaf, stem and root of a transgenic plant and no GUS expression in the wild-type (WT). **e**PCR analysis of *StSnRK1*-overexpressing tobacco plants. Lane M: DL2000 DNA marker; Lane W: water as negative control; Lane P: plasmid pCAMBIA1301-*StSnRK1* as positive control; Lane WT: wild type; Lanes #1-#6: different transgenic lines

Fig. 5 Expression analysis of the *StSnRK1* gene in the transgenic tobacco plants by real-time quantitative PCR. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. The tobacco *Ntactin* gene was used as an internal control. Data are presented as mean $\pm$ SE ($n = 3$). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's *t*-test

Fig. 6 SnRK1 activity assay in the leaves of WT and transgenic plants. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. Data are presented as mean $\pm$ SE ($n = 3$). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's *t*-test

Fig. 7 Sucrose, glucose, fructose and soluble sugar content analyses in the leaves of WT and transgenic plants. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. Data are presented as mean $\pm$ SE (n = 3). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's *t*-test

Fig. 8 Starch content analysis in the leaves of WT and transgenic plants. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. Data are presented as mean $\pm$ SE (n = 3). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's *t*-test

tubers to sucrose supply (Tiessen et al. 2003; Geigenberger et al. 2005).

The *SnRK1* was involved in the control of *SUS* gene in potato and it has been shown that *SnRK1* regulate *SUS* transcription and thus affects various aspects of carbon metabolism in plants (Purcell et al. 1998). *SnRK1* can regulate carbohydrate metabolism by phosphorylating and inactivating *SPS* (Sugden et al. 1999 and Halford and Hey 2009). The study found that the expression of *SUS* and *AGPase* was up-regulated and their enzyme activities were also increased in the *SnRK1*-overexpressing potato plants, in which starch levels in the tubers were increased by up to 30%, while the expression of gene encoding *SPS* showed no change from the wild-type (McKibbin et al. 2006). Wang et al. (2012) and Jiang et al. (2013) demonstrated that overexpression of *SnRK1* from apple/sweet potato up-regulated the expression of *SUS* and *AGPase*, and increased the activities of *SUS* and *AGPase* and decreased the activity of *SPS*, leading to increased soluble sugar and

Fig. 9 Southern blot analysis of the transgenic plants to detect the copy number of integrated *StSnRK1* gene. WT, wild type; #2, #3 and #4, transgenic plants with higher starch content

Fig. 10 Transcript levels of starch biosynthesis genes in the leaves of WT and transgenic plants. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. The tobacco *Ntactin* gene was used as an internal control. Results are expressed as relative values with respect to WT, which was set to 1.0. Data are presented as mean $\pm$ SE (n = 3). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's *t*-test

starch content in transgenic tomato/tobacco plants. In our work, the expression of *NtSUS* and *NtAGPase* was up-regulated, while the expression of *NtSPS* was slightly inhibited in transgenic tobacco plants (Fig. 10). Consistently, enzymatic activity of *SUS* and *AGPase* were also significantly increased, and *SPS* activity was also decreased in the *StSnRK1*-overexpressing tobacco plants (Fig. 11).

Soluble starch synthase (*SSS*) is responsible for the synthesis of starch in plants (Delvallé et al. 2005; Ren et al. 2007; Geigenberger 2011). Wang et al. (2016a, c, d; 2017a, b) reported that the activated starch biosynthesis in the *ZmTrxF/SIAATP/SITrxF/StAATP/StTrxF*-overexpressing *Arabidopsis*

Fig. 11 SUS, SPS, AGPase and SSS enzyme activity assays in the leaves of WT and transgenic plants. The transgenic plants and WT were grown in pots under normal conditions for 4 weeks. Data are presented as mean $\pm$ SE ($n = 3$). * and ** indicate a significant difference from that of WT at $P < 0.05$ and < 0.01 , respectively, by Student's t -test

Fig. 12 A proposed model of the regulatory network of *StSnRK1* in starch accumulation. The pathway for carbon destined for conversion to starch is shown with solid arrows and regulatory interactions are shown with broken arrows

plants was related to the enhanced enzyme activity of SSS and the increased expression of *StSSS* involved in starch biosynthesis. In our study, the activity of SSS was increased and the expression of *NtSSS III* was up-regulated in the *StSnRK1*-overexpressing tobacco plants (Figs. 10, 11). Thus, it is thought that overexpression of *StSnRK1* up-regulates the expression of *NtSUS* and *NtAGPase* and increases the activities of SUS and AGPase, which further increase the expression of the genes and enhance the activity of the major enzymes related to starch biosynthesis, leading to increased starch accumulation in transgenic plants (Fig. 12).

The presence of sufficient substrate (sucrose) is a very important factor in the starch biosynthesis pathway. The studies found that the expression of *SUS* and *AGPase* was induced by sucrose in potato tubers (Müller-Röer et al. 1990; Fu and Park 1995). In our work, the increased content of sucrose was observed (Fig. 7), and the up-regulation of *NtSUS* and *NtAGPase* genes was also found in transgenic tobacco plants (Fig. 10). It is thought that the high

level of sucrose can induce the expression of *NtSUS* and *NtAGPase*, further modulating starch metabolism in transgenic plants (Fig. 12). All of the findings suggest that *StSnRK1* transfers carbon through the storage pathway to starch by regulating the activities of SUS, SPS and AGPase. Our results support the hypothesis proposed by Halford and Hey (2009) and Jiang et al. (2013) that SnRK1 is activated by sucrose, and then *SnRK1* increases flux through the starch biosynthesis pathway by up-regulating the expression of *NtSUS* and *NtAGPase* and down-regulating the expression of *NtSPS* (Fig. 12).

In addition, the present results indicate that there was no clear relationship between the starch accumulation and the copy number of integrated *StSnRK1* gene, similar to the results reported by Wang et al. (2016b), in which the copy number of integrated *SLAATP* gene ranged from 1 to 2 in transgenic plants exhibiting higher starch content.

Taken together, the *StSnRK1* gene was successfully isolated from potato. Overexpression of *StSnRK1* in tobacco showed significant increase in soluble sugar and starch content. Our results suggest that *StSnRK1* plays an important role in starch metabolism, and has great impact in the engineering of alternative energy crop plants with improved starch accumulation.

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