A tomato plastidic ATP/ADP transporter gene *SlAATP* increases starch content in transgenic *Arabidopsis*

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Abstract A plastidic ATP/ADP transporter (AATP) is responsible for importing ATP from the cytosol into plastids. Increasing the ATP supply is a potential way to facilitate anabolic synthesis in heterotrophic plastids of plants. In this work, a gene encoding the AATP protein, named *SlAATP*, was successfully isolated from tomato. Expression of *SlAATP* was induced by exogenous sucrose treatment in tomato. The coding region of *SlAATP* was cloned into a binary vector under the control of 35S promoter and then transformed into *Arabidopsis* to obtain transgenic plants. Constitutive expression of *SlAATP* significantly increased the starch accumulation in the transgenic plants. Real-time quantitative PCR (qRT-PCR) analysis showed that constitutive expression of *SlAATP* up-regulated the expression of phosphoglucomutase (*AtPGM*), ADP-glucose pyrophosphorylase (*AtAGPase*), granule-bound starch synthase (*AtGBSS I* and *AtGBSS II*), soluble starch synthases (*AtSSS I*, *AtSSS II*, *AtSSS III* and *AtSSS IV*) and starch branching enzyme (*AtSBE I* and *AtSBE II*) genes involved in starch biosynthesis in the transgenic *Arabidopsis* plants. Meanwhile, enzymatic analyses indicated that the major enzymes (AGPase, GBSS, SSS and SBE) involved in the starch biosynthesis exhibited higher activities in the transgenic plants compared to the wild-type (WT). These findings suggest that *SlAATP* may improve starch content of *Arabidopsis* by up-regulating the

expression of the related genes and increasing the activities of the major enzymes involved in starch biosynthesis. The manipulation of *SlAATP* expression might be used for increasing starch accumulation of plants in the future.

Keywords *Arabidopsis* · Constitutive expression · *SlAATP* · Starch content · Tomato

Introduction

Biofuels have the potential to reduce our dependence on fossil fuels as well as reduce the environmental damage caused by their extraction and use. A search for nonfood energy crops for the economically viable production of environmentally friendly biofuels is therefore underway (Sanz-Barrio et al. 2013). As conversion of starch into fermentable sugars is relatively easy, starch has been taken as a major feedstock for first-generation biofuel production (Smith 2008; Sanz-Barrio et al. 2013). Therefore, it is vital to know how carbohydrates are metabolized in plants, which could be of great help in the development of crops by means of enhancing starch synthesis, and in the improvement of biofuel production efficiency (Sanz-Barrio et al. 2013).

In plants, starch is an insoluble glucan composed of two glucose polymers: amylose and amylopectin. Amylose is mainly comprised of linear chains that are linked by α -1,4 O-glycosidic bonds, whereas amylopectin is highly branched and contains 5–6% α -1,6 O-glycosidic bonds to generate glucan branches of various lengths (Delvallé et al. 2005). Four major enzymes are involved in starch biosynthesis: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE) and starch debranching enzyme (DBE) (Fujita et al. 2006).

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In below-ground storage organs, the best option to increase the starch content may lie in increasing the ATP supply to the plastid (Smith 2008). ATP is the basic energy currency in living cells and is needed for almost every step of biochemical reactions. ATP is also an indispensable participant in the AGPase reaction, which catalyzes the formation of ADP-glucose (ADPG) and is considered as a rate-limiting enzyme in starch biosynthesis (Jeon et al. 2010). ATP exchanges between organelles and the cytosol is mediated by adenylate carrier proteins. One type of adenylate carrier protein is the mitochondrial ADP/ATP carrier (AAC), which can export ATP produced previously via oxidative phosphorylation in a one to one exchange of cytosolic ADP (Fiore et al. 1998). The mitochondrial AAC is a dimer, and each monomer contains six transmembrane helices (Winkler and Neuhaus 1999). Another type of adenylate carrier protein is the plastidic ATP/ADP transporter protein (AATP), which was discovered in spinach chloroplasts. AATP is generally found in the heterotrophic plastids (amyloplasts, chromoplasts and leucoplasts) of higher and lower land plants as an important energy transporter (Heldt 1969; Schünemann et al. 1993; Emes and Neuhaus 1997; Möhlmann et al. 1998; Linka et al. 2003; Meng et al. 2005; Yuen et al. 2009). The main function of AATP is to provide the plastid stroma with cytosolic ATP, which is required for anabolic processes such as starch and fatty acid synthesis (Möhlmann et al. 1998).

There are a few studies about the functions of AATP in regulating starch biosynthesis in plants. Constitutive expression of the *Arabidopsis AtAATP1* increased ADPG level up to twofolds and starch content by 16–36% (Tjaden et al. 1998). In contrast, the antisense inhibition of the potato *StAATP* decreased ADPG level by 25–70% and starch content by 19–51% in potato tubers (Geigenberger et al. 2001). In another report, down-regulation of the plastidic adenylate kinase (*SlAK*) gene, interconverting ATP and AMP into ADP, caused a substantial effect on the adenylate pool size and the ADPG level in potato tubers, further increasing the starch content (Regierer et al. 2002). These results indicate that the manipulation of the enzymes that modulate the ATP supply in plastids is an effective way to enhance starch biosynthesis in plants.

Although the function of AATP from other plant species has been well studied, the regulatory role of *SIAATP* (Genbank accession No. XP_004235723) from tomato in increasing starch accumulation still remains unknown. In this study, we isolated *SIAATP* from tomato and estimated its roles in transgenic *Arabidopsis*. Ectopic expression of *SIAATP* significantly increased the starch accumulation in the leaves of transgenic plants. Our results indicate that *SIAATP* is a promising candidate gene for increasing starch content in plants.

Materials and methods

Plant materials

Tomato cultivar Zhongshu No. 4, an important tomato cultivar in China, was employed for *SIAATP* gene cloning in this study. *Arabidopsis* [ecotype Columbia-0, wild type (WT)] was used as a model plant to investigate the functions of *SIAATP*.

Cloning of the tomato *SIAATP* gene

Total RNA was extracted from the leaves of Zhongshu No. 4 with the RNeasy Pure Kit (Qiagen Biotech, Beijing, China). RNA samples were reverse-transcribed according to the instructions of Quantscript Reverse Transcriptase Kit (Qiagen Biotech, Beijing, China). Based on the sequence of *SIAATP* (Genbank accession No. XP_004235723), we designed one gene-specific primers (GC-F/R) of reverse transcription PCR (RT-PCR) (Table S1) to obtain its full-length cDNA sequence. The genomic sequence of *SIAATP* was amplified with primers GA-F/R (Table S1) using genomic DNA extracted from the leaves of Zhongshu No. 4 as a template.

Sequence analysis of the *SIAATP* gene

The open-reading frame (ORF) of the cloned *SIAATP* gene was predicted with ORF Finder (<http://www.ncbi.nlm.nih.gov/projects/gorf/>). The homology of *SIAATP* protein was identified using protein BLAST in the National Center for Biotechnology Information (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The conserved domain of *SIAATP* protein was scanned by the InterProScan program (<http://www.ebi.ac.uk/Tools/pfa/iprscan/>). The theoretical molecular weight and isoelectric point (*pI*) were calculated using ProtParam tool (<http://web.expasy.org/protparam/>). The genomic structure of *SIAATP* was analyzed using the Spidey Program (<http://www.ncbi.nlm.nih.gov/spidey/>). The transmembrane helices in the *SIAATP* protein were detected using TMHMM Server (v. 2.0, <http://www.cbs.dtu.dk/services/TMHMM/>). For multiple sequence alignment analysis, the amino acid sequences of *SIAATP* and other AATP homologs from different plant species retrieved from NCBI were aligned using the DNAMAN software (Lynnon Biosoft, Quebec, Canada). Phylogenetic analysis was conducted with the MEGA4 software (<http://www.megasoftware.net/>).

Expression analysis of *SIAATP* in tomato

The response of *SIAATP* to exogenous sucrose was investigated based on the method of Wang et al. (2001) with some modifications. Leaves from Zhongshu No. 4 were

supplied with water (control) or 175 mM sucrose in darkness at 28 °C after being cultured in water in the dark for 1 day. Real-time quantitative PCR (qRT-PCR) was conducted to determine the transcript levels of *SIAATP* in the leaves at different time points (0, 3, 6, 12, 24 and 48 h) after treatment using SYBR Green PCR Master Mix (Tiangen Biotech, Beijing, China) and ABI PRISM 7500 (Software for 7500 and 7500 Fast Real-Time PCR Systems, V2.0.1, USA). Total RNA was isolated using RNeasy Pure Plant Kit (Tiangen Biotech, Beijing, China) and first-strand cDNA was prepared by Quantscript Reverse Transcriptase Kit (Tiangen Biotech, Beijing, China). The primers used to amplify *SIAATP* were listed in Table S1. The tomato *Slactin* gene (Genbank accession No. BT013524) was used as an internal control (Loukehaich et al. 2012) (Table S1).

Generation of transgenic *Arabidopsis* plants

The coding region of *SIAATP* was amplified using a pair of specific primers with terminal *Bam*H I and *Sac* I restriction sites, and then inserted into the same enzyme sites in pCAMBIA1301 to create the plant expression vector pCAMBIA1301-*SIAATP*, under the control of the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) terminator. This vector also contains β -glucuronidase (*gusA*) and hygromycin resistance (*hpt* II) genes driven by the CaMV 35S promoter. Both pCAMBIA1301-*SIAATP* and the control vector (VC) pCAMBIA1301 were transformed into the *Agrobacterium tumefaciens* strain LBA4404 cells by the electroporation method for *Arabidopsis* transformation (Lou et al. 2007). Transgenic plants were produced according to methods described previously (Zhang et al. 2006). Transformants were selected based on their resistance to hygromycin (Hyg). Putative transformant seeds were germinated on agar-solidified MS (Murashige and Skoog 1962) medium containing 25 mg/L Hyg. Positive transgenic seedlings were grown in pots containing a mixture of soil, vermiculite and humus (1:1:1, v/v/v) for T₂ and T₃ seed selection. The incubation and growth conditions of *Arabidopsis* were the same as described previously (Zhang et al. 2006).

Molecular confirmation of transgenic plants

The presence of *SIAATP* in hygromycin-resistant plants was assessed by PCR analysis using specific primers (Table S1) to amplify fragments of the *hpt* II coding sequence. DNA was first extracted from *Arabidopsis* leaves according to the instructions of EasyPure Plant Genomic DNA Kit (Transgen, Beijing, China). PCR amplifications were performed with an initial denaturation 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 final extension 72 °C for 10 min. PCR products were separated by electrophoresis on a 1.0% (w/v) agarose gel.

Starch content assays

Starch extraction and quantification were performed as described previously (Smith and Zeeman 2006). Seeds were grown on MS medium for 2 weeks and transferred to pots containing a mixture of soil, vermiculite and humus (1:1:1, v/v/v). Plants were grown in growth chamber for 4 weeks at 22 °C under standard long day conditions (14 h light and 10 h dark). Leaves of four-week-old plants were harvested to determine 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, VC and WT plants by cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1985). Approximately 20 μ g genomic DNA of each sample was digested by *Eco*R I. The restriction fragments were size-fractionated by 1.0% (w/v) agarose gel electrophoresis and transferred to a Hybond-N+ nylon membrane (Amersham Pharmacia Biotech, UK). A 560 bp *SIAATP* fragment coding sequence generated with specific primers (Table S1) was used as the probe. The labeling of probe, prehybridization, hybridization and detection were performed by the protocol of DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics GmbH, Germany).

Expression analysis of the related genes

The expression of *SIAATP* and starch biosynthesis related genes was analyzed by qRT-PCR. Transgenic, VC and WT plants were grown in pots for 4 weeks under normal condition. Total RNA was extracted from the leaves of these plants, respectively, using the RNeasy Pure Plant Kit (Tiangen Biotech, Beijing, China). RNA samples were reverse-transcribed using Quantscript Reverse Transcriptase Kit (Tiangen Biotech, Beijing, China). The cDNA solution was used as templates for PCR amplification with gene specific primers (Table S1). *Arabidopsis Atactin* gene (Genbank accession No. NM112764) was used as an internal control (Li et al. 2013) (Table S1).

AGPase, GBSS, SSS and SBE activity analyses

The activity of four starch biosynthetic enzymes (AGPase, GBSS, SSS and SBE) in the leaves of four-week-old transgenic, VC and WT plants was performed according to the method described by Nakamura et al. (1989). One unit of

enzyme activity (AGPase, GBSS and SSS) was defined as the formation of 1 nmol ADP per min at 30 °C and 1 unit of SBE activity was defined as the amount of enzyme required to increase the spectrophotometric absorbance by 1 unit in 1 min.

Statistical analyses

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 analyses of *SIAATP*

The *SIAATP* (Genbank accession No. XP_004235723) gene was cloned by RT-PCR. *SIAATP* contained a 1878 bp ORF, encoding a polypeptide of 625 amino acids, with a molecular weight of 68.38 kDa and a theoretical isoelectric point (*pI*) of 9.37. Sequence analysis via the InterProScan program (<http://www.ebi.ac.uk/Tools/pfa/iprscan/>) showed that the *SIAATP* protein contained an ADP/ATP carrier protein domain (Fig. 1a). The genomic sequence of *SIAATP* was 3295 bp long and contained 5 exons and 4 introns (Fig. 1b). According to the online software TMHMM (v. 2.0), *SIAATP* contains 10 transmembrane (TM) helices (Fig. 1c), which is one of the main structural characteristics of the AATP family (Winkler and Neuhaus 1999; Yuen et al. 2009).

The deduced amino acid sequence alignment showed that *SIAATP* was highly conserved and homologous to the AATPs from *Arabidopsis thaliana* (AtAATP1, 2), *Mentha spicata* (MsAATP), *Oryza sativa* (OsAATP1, 2), *Solanum tuberosum* (StAATP) and *Zea mays* (ZmAATP), especially in the TM regions (Fig. 2). Further homology analyses using DNAMAN showed that *SIAATP* had 66.98–87.50% amino acid identity to ZmAATP, OsAATP1, OsAATP2, AtAATP2, AtAATP1, MsAATP and StAATP (Fig. 2). Five highly conserved motifs [FLKT, AELWG, FANQIT, AYG(I/V)S(I/V)NLVE and (L/I)GKSGGA(L/I)IQ] present in the plant and bacterial ATP/ADP transporter proteins were also identified (Fig. 2), indicating the importance of these motifs in determining AATP function (Möhlmann et al. 1998; Meng et al. 2005). Phylogenetic analyses revealed that *SIAATP* had a close relationship with StAATP (Fig. 3).

Expression analysis of *SIAATP* in tomato

To examine whether the expression of *SIAATP* responded to exogenous sucrose, we floated young leaves in water

Fig. 1 Gene structure of plastidic ATP/ADP transporter protein (*SIAATP*) from *Solanum lycopersicum*. **a** Structure of *SIAATP* and *SIAATP*. The *SIAATP* protein contains an ADP/ATP carrier protein domain. **b** Exon/intron organization of *SIAATP* gene with location of exons (brown boxes) and introns (blue lines). **c** Prediction of transmembrane helices in the *SIAATP* protein (color figure online)

(control) or 175 mM sucrose for up to 48 h. The results showed that no induction of the *SIAATP* transcript occurred when the leaves were treated with water as control (Fig. 4). In contrast, the presence of exogenous sucrose could significantly enhance the accumulation of the *SIAATP* transcript, reaching the highest level (1.60-folds) at 12 h and declining thereafter (Fig. 4).

Increased starch content in *Arabidopsis* expressing *SIAATP*

The ORF of *SIAATP* was ectopically expressed in *Arabidopsis* (Col-0, WT) using the binary vector pCAMBIA1301-*SIAATP* (Fig. 5a). Ten independent transgenic lines constitutively expressing *SIAATP* (T_1 generation) were obtained by Hyg resistance selection, named #1–#10, respectively, and their progenies (T_3 generation) were generated. PCR analyses of genomic DNA confirmed the successful integration of transgene (Fig. 5b). qRT-PCR analyses showed that the highest expression levels of *SIAATP* were observed in transgenic lines #3, #8 and #10, while no transgene expression was observed in VC and WT (Fig. 5c). Therefore, transgenic lines #3, #8 and #10 were selected for further analyses.

Two-week-old WT, VC and transgenic plants (lines #3, #8 and #10) were grown in pots under normal condition for 4 weeks. Expression of *SIAATP* did not change the growth of transgenic plants since no significant differences in growth was

Fig. 2 Multiple sequence alignment of AATP proteins from *Solanum lycopersicum* (SIAATP), *Arabidopsis thaliana* (AtAATP1, 2), *Mentha spicata* (MsAATP), *Oryza sativa* (OsAATP1, 2), *Solanum tuberosum* (StAATP) and *Zea mays* (ZmAATP). The ten transmembrane helices of

StAATP predicted by TMHMM are outlined. Five highly conserved motifs (FLTK, AELWG, FANQIT, AYGI(I/V)NLVE and (L/I)GKSGGA(L/I)IQ) present in the plant and bacterial ATP/ADP transporter proteins are indicated in yellow boxes (color figure online)

Fig. 3 Phylogenetic analysis of plastidic ATP/ADP transporters from *Solanum lycopersicum* (SIAATP) and other plant species. Sequences were from *Solanum tuberosum* (StAATP), *Mentha spicata* (MsAATP), *Arabidopsis thaliana* (AtAATP1, 2), *Oryza sativa* (OsAATP1, 2) and *Zea mays* (ZmAATP)

observed between WT, VC and the transgenic plants under normal conditions. However, the starch content in the leaves of these plants was different. The starch content in SIAATP expressing plants increased 48–87% compared to that in WT (Fig. 6), whereas no significant difference was observed between VC and WT plants (Fig. 6).

Fig. 4 Effect of exogenous sucrose treatment on SIAATP transcript accumulation in leaves. The results were expressed as relative values with respect to 0 h, which were set to 1.0. The tomato *actin* gene was used as an internal control. Data are presented as the mean $\pm$ SE (n = 3)

Southern blot analysis of Arabidopsis expressing SIAATP

Transgene integration patterns of the 3 transgenic plants (lines #3, #8 and #10) with higher starch content were analyzed by Southern blot. The genomic DNA of the transgenic plants, VC and WT was digested with *EcoR* I,

Fig. 5 Molecular confirmation of transgenic plants. **a** Schematic diagram of the T-DNA region of binary plasmid pCambia1301-SIAATP. LB, left border; RB, right border; hpt II, hygromycin phosphotransferase II gene; SIAATP, tomato plastidic ATP/ADP transporter protein gene; gusA, β -glucuronidase gene; 35S, cauliflower mosaic virus (CaMV) 35S promoter; 35S T, CaMV 35S terminator; NOS T, nopaline synthase terminator. **b** PCR analysis of SIAATP expressing *Arabidopsis* plants. Lane M DL2000 DNA marker, Lane W water as negative control, Lane P plasmid pCambia1301-SIAATP as positive control, Lane WT wild type, VC control vector, Lanes #1–#5 different transgenic lines. **c** Expression levels of SIAATP in different transgenic lines. The *Arabidopsis actin* 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 Starch content analyses in the leaves of WT and transgenic plants. Four-week-old WT and transgenic plants grown under normal condition were used. 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

which has a unique cleavage site in the T-DNA region in the vector and hybridized with the SIAATP gene probe. The transgenic plants displayed different patterns and the copy number of integrated SIAATP gene varied from 1 to 2

(Fig. 7). No hybridizing band was observed in WT and VC as expected (Fig. 7). Clear relationship between the starch accumulation and the copy number was not also found.

Up-regulation of starch biosynthetic genes in *Arabidopsis* expressing SIAATP

To dissect how expression of SIAATP increased starch content in transgenic plants, the transcript levels of 13 starch biosynthetic genes in WT, VC and transgenic plants (lines #3, #8 and #10) were examined by qRT-PCR (Fig. 8). Expression of the genes related to starch biosynthesis pathway, such as phosphoglucomutase (*AtPGM*), AGPase small subunit (*AtAGPase-S1* and *AtAGPase-S2*), AGPase large subunit (*AtAGPase-L1* and *AtAGPase-L2*), granule-bound starch synthase (*AtGBSS I* and *AtGBSS II*), soluble starch synthases (*AtSSS I*, *AtSSS II*, *AtSSS III* and *AtSSS IV*) and starch branching enzyme (*AtSBE I* and *AtSBE II*) was all up-regulated in transgenic plants (Fig. 8). These results indicate that SIAATP might be involved in the regulation of starch biosynthetic processes.

Enhanced enzyme activities in *Arabidopsis* expressing SIAATP

The activity of major enzymes (AGPase, GBSS, SSS and SBE) involved in starch biosynthesis was also investigated in the leaves of WT, VC and transgenic plants (lines #3, #8 and #10) (Fig. 9). The results showed that the activity of these enzymes was significantly enhanced in transgenic plants compared to that in the WT (Fig. 9). The increases in enzyme activity in transgenic plants were consistent with the increased transcription levels of their corresponding

Fig. 7 Southern blot analysis of the transgenic plants to detect the copy number of integrated SIAATP gene. WT wild type; VC control vector; #3, #8 and #10 transgenic plants with higher starch content

Fig. 8 Transcript levels of starch biosynthesis genes in the leaves of WT and transgenic plants. Four-week-old WT and transgenic plants grown under normal condition were used. The *Arabidopsis actin* 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

genes. All these results demonstrate that *SIAATP* has significant effects on the activities of AGPase, GBSS, SSS and SBE in transgenic *Arabidopsis*.

Discussion

Starch is the major dietary source of carbohydrates and the most abundant storage of polysaccharide in higher plants. The *AATP* gene has been shown to be involved in starch accumulation in plants. The *Arabidopsis* expressing

AtAATP1 exhibited increased ADP-glucose level up to twofold and starch content by 16–36% (Tjaden et al. 1998). And the antisense inhibition of the potato *StAATP* decreased ADPG level by 25–70% and starch content by 19–51% in potato tubers (Geigenberger et al. 2001).

In our work, we isolated the *SIAATP* gene from tomato, and sequence analysis indicated that *SIAATP* is a previously unreported member of the plastidic ATP/ADP transporter gene family (Figs. 1, 2, 3). We also found that the transcript level of *SIAATP* was induced by exogenous sucrose treatment in tomato, indicating that *SIAATP* may

Fig. 9 AGPase, GBSS, SSS and SBE enzyme activity assays in the leaves of WT and transgenic plants. Four-week-old WT and transgenic plants grown under normal condition were used. 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

be tightly associated with starch biosynthesis in tomato and may serve as a means to reprogram chloroplasts into starch accumulating ATP-importing storage plastids (Koch 1996; Reiser et al. 2004). Once the sugar level in the cytosol is higher than actually needed, *SlAATP* will be strongly induced, which couples this high sugar level by importing more ATP into the plastids, thus triggering the starch biosynthesis (Fig. 4). Our work indicated that constitutive expression of *SlAATP* significantly increased the starch accumulation in the transgenic *Arabidopsis* plants (Fig. 5).

In heterotrophic organs, the carbon precursors and ATP needed for anabolic processes are mainly imported from the cytoplasm (Emes and Neuhaus 1997; Winkler and Neuhaus 1999). The levels of ATP or ADP-glucose (ADPG) are key targets for genetic manipulation when attempting to accelerate starch biosynthesis in amyloplasts. ATP uptake by amyloplasts is mediated by a plastidic ATP/ADP transporter (AATP) (Schünemann et al. 1993). Constitutive expression of *AtAATP1* in potato resulted in the more imported ATP levels from the cytosol into the stroma, which facilitated the synthesis of ADPG, leading to an increase in starch accumulation in tubers (Tjaden et al. 1998). In the current study, the starch content in the transgenic plants was significantly enhanced (Fig. 5). Expression of *SlAATP* could also increase ATP import levels into amyloplasts, energizing the pivotal AGPase reaction in starch biosynthesis (Fig. 10). We found that the expression of *AtAGPase-S1* and *AtAGPase-S2*, encoding AGPase small subunit, and *AtAGPase-L1* and *AtAGPase-L2*, encoding AGPase large subunit, was up-regulated in transgenic plants (Fig. 8). Consistently, enzymatic activity of AGPase was also significantly increased in the *SlAATP* expressing *Arabidopsis* plants (Fig. 9). These results showed that ADPG, as a large amount of the ultimate precursor for starch synthesis, could be more accumulated (Fig. 9). Meanwhile, the consumption of glucose-1-phosphate (G-1-P) in the AGPase reaction required the

Fig. 10 A proposed model of the regulatory network of *SlAATP* in starch accumulation. Biosynthesis pathway is shown with solid arrows and regulatory interactions with broken arrows. Upwards arrow indicates up-regulation of the relative enzyme encoding genes

accelerated conversion of glucose-6-phosphate (G-6-P) to G-1-P, which was catalyzed by phosphoglucumutase (PGM) (Harrison et al. 2000), and consequently, the transcription of *AtPGM* was up-regulated (Fig. 8). These results suggest that expression of *SlAATP* enhanced the starch biosynthesis due to the increased ATP supply into amyloplasts, which further increased the production of precursors (ADPG and G-1-P) and the expression of starch biosynthesis related genes (Fig. 10).

The higher level of starch content is related to the increased expression of starch biosynthesis genes (Delvallé et al. 2005; Jiang et al. 2013). Starch synthase can be grouped into five types, granule-bound starch synthase (GBSS) and four types of soluble starch synthases (SSS): SSS I, SSS II, SSS III and SSS IV (Delvallé et al. 2005; Fujita et al. 2006; Szydlowski et al. 2009). A large body of evidence has illustrated that up-regulation of these genes could increase starch accumulation in plants (Burton et al. 2002; Bustos et al. 2004; Roldan et al. 2007; Szydlowski et al. 2009; Jiang et al. 2013). In our study, the activities of the major enzymes (GBSS, SSS and SBE) involved in starch biosynthesis were increased in transgenic plants (Fig. 9). Consistent with this phenomenon, systematic up-regulation of these genes (*AtGBSS I*, *AtGBSS II*, *AtSSS I*,

AtSSS II, *AtSSS III*, *AtSSS IV*, *AtSBE I* and *AtSBE II*) involved in starch biosynthesis pathway was also observed in transgenic plants (Fig. 8). Therefore, it is thought that constitutive expression of *SIAATP* increases the expression of the genes and the activity of the major enzymes involved in starch biosynthesis, resulting in the improved starch accumulation in transgenic plants (Fig. 10).

In conclusion, the *SIAATP* gene was successfully isolated from tomato. Constitutive expression of *SIAATP* significantly increased starch accumulation in transgenic *Arabidopsis* plants by the up-regulation of starch biosynthetic related genes. Our results suggest that *SIAATP* plays a crucial role in starch metabolism, and has great potential in the engineering of alternative energy crop plants with improved starch accumulation.

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