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Original Paper

Isolation and characterisation of the ADP-glucose pyrophosphorylase small subunit gene (*AgpS1*) promoter in tomato (*Solanum lycopersicum* L.)

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Abstract ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis. In this research, 2,885 bp of the predicted promoter sequence for the *AgpS1* gene encoding the AGPase small subunit was isolated from tomato. Sequence analyses revealed a number of known *cis*-elements related to responses to salt and dehydration stress and sugar repression; predicted TATA boxes are located at −88 to −94 bp and −114 to −120 bp. The spatial expression pattern and tissue/organ specificity of *AgpS1* were analysed in during development using promoter-GUS transgenic tomato plants. Based on GUS staining, the obtained sequence was proven to be the functional promoter and directed broad expression in both sink and source tissues/organs, including seedling, stem, flower, fruit stalk, fruit and root. In source leaf and early developing fruit, GUS staining was observed in all tissues, except for epidermal tissue. In contrast, GUS staining tended to be confined to vascular tissues in seedling, stem, fruit stalk and ripening fruit. In particular, a patchy staining pattern was observed in the phloem of the stem and fruit stalk, suggesting that *AgpS1* is expressed in the phloem companion cells in those organs. These results also suggest that AGPase mainly functions in the vascular tissue of those organs.

Key words: ADP-glucose pyrophosphorylase, *AgpS1*, promoter, tomato.

The sugar content is one of most important properties determining the taste and quality of fruit. It has been reported that starch accumulation during early development in tomato fruit affects the sugar level of red-ripe fruit (Baxter et al. 2005; Petreikov et al. 2006; Yin et al. 2010). ADP-glucose pyrophosphorylase (AGPase, EC2.7.7.27) catalyses the first regulatory step in starch biosynthesis in plants (Lin et al. 1988; Müller-Röber et al. 1992; Stark et al. 1992; Tsai and Nelson 1966): the synthesis of ADP-glucose from glucose-1-phosphate and ATP (Preiss 1988). Plant AGPase is a hetero-tetrameric enzyme composed of two small and two large subunits (Morell et al. 1987). The former subunits function as the catalytic molecule, and the latter subunits function as allosteric modulators (Okita et al. 1990).

In tomato, one gene encoding the small subunit and three genes encoding the large subunit have been identified thus far (Chen et al. 1998; Park and Chung, 1998), with *AgpS1* and *AgpL1*, encoding a small and large subunit, are predominantly expressed and are responsible for starch accumulation in early developing fruit (Park and Chung 1998; Petreikov et al. 2006; Yin et al. 2010). It has been reported that plant AGPase genes are regulated at the transcriptional level by phosphate, nitrate and sugars (Li et al. 2002; Müller-Röber et al. 1990; Nielsen et al. 1998; Scheible et al. 1997; Sokolov et al. 1998). Additionally, our previous research revealed that *AgpS1* and *AgpL1* were specifically upregulated at the transcriptional level by salinity stress in early developing fruits in an ABA- and osmotic stress-independent manner (Yin et al. 2010). In fact, the *AgpL1* response to salinity was a sugar-mediated response due to the elevated carbohydrate influx into the fruit under the salinity stress (Yin et al. 2010).

Early works in potato, *Arabidopsis* and tomato have reported differential regulation among the AGPase genes in terms of responses to sugars and light and in sink and source tissues (Müller-Röber et al. 1990; Nakata et al.

Abbreviations: AGPase, ADP-glucose pyrophosphorylase; GUS, *β*-glucuronidase, *P35S*, cauliflower mosaic virus 35S promoter; X-Gluc, 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid cyclohexylammonium salt.

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1995; Sokolov et al. 1998). However, in contrast to the results of Li et al. (2002), who showed that the expression of both *AgpL1* and *AgpS1* (referred to as *AgpS1* and *AgpB*, respectively) were induced in fruit by sucrose at the transcriptional level, a clear response was not observed in our study (Yin et al. 2010). At present, there is less information regarding the regulation of the gene for the small subunit than those for the large subunits. Indeed, the spatial expression pattern of the *AgpS1* gene in tomato has not been clarified to date.

A promoter analysis is one of the most useful ways to clarify spatial expression pattern and regulation manner of a target gene. Although the promoter sequences of *AgpL1* and *AgpL3* have been isolated and analysed in tomato (Xing et al. 2005), information of the *AgpS1* gene promoter is still lacking. Therefore, in the present work, we isolated 2,885 bp of the sequence upstream of the *AgpS1* gene and analysed the spatial expression pattern using a promoter-GUS transgenic plant in tomato. Sequence analyses revealed there are many predicted *cis*-elements relevant to response to abiotic stresses and sugar-repression in the promoter sequence. GUS staining assay revealed the different tissue specificity of *AgpS1* expression in different organs in tomato plant.

Materials and methods

Preparation of the walking PCR library and isolation of the AgpS1 promoter region

The walking PCR library was prepared using tomato (*Solanum lycopersicum* L. cv., 'Micro-Tom') genomic DNA extracted from leaves with the BD Genome Walker Universal Kit (Clontech, Mountain View CA, USA). The Genome Walker Adaptor was ligated to the genomic DNA according to the manufacturer's instructions. The adaptor sequences were 3′-H_2N-CCCGACCA-PO_4-5′ (ADS, 8-mer) and 5′-GTA ATA CGA CTC ACT ATA GGG CAC GCG TGG TCG ACG GCC CGG GCT GGT-3′ (ADL, 48-mer). ADS and ADL were mixed in the proportion of one part to six and incubated for 5 min at 65°C and then for 10 min at 37°C for annealing. A 2-μg sample of genomic DNA was digested with *Stu*I at 37°C for 18 h. The digested genomic DNA was purified by ethanol precipitation, and the Genome Walker Adaptor was ligated to the DNA fragments using T4 ligase (Promega, Madison, WI, USA), which was used as the Walking PCR library.

Isolation of the *AgpS1* promoter region was performed in two steps. First, we searched the 5′-upstream region of *AgpS1* constructive gene using the tomato genome DNA data base provided from the SOL Genomics Network (SGN, http://solgenomics.net/) (The Tomato Genome Consortium, 2012) and identified a sequence including 2,051 bp of the upstream region from the start ATG codon. According to the sequence, two primers, Rev3 at −1,072 to −1,099 bp (5′-GTC CAT CCC TGG CAC ACT GCG GTG CAA G-3′) and Rev4 at −1,383 to −1,408 bp (5′-CCA CCT GAA GGC CCC GGA ATA CCT CC-3′), were designed as the walking PCR primers (Figure S1). Second, the walking PCRs were performed used to amplify the walking library DNA with the ADL sequence-specific primers, AP1 (5′-GTA ATA CGA CTC ACT ATA GGG C-3′) and AP2 (5′-ACT ATA GGG CAC GCG TGG T-3′), and Rev3 and Rev4 primers. The initial PCR reaction was performed with the AP1 and Rev3 primers and the following PCR conditions: 7 cycles of 94°C for 25 s and 72°C for 3 min, 32 cycles of 94°C for 25 s and 67°C for 3 min and 67°C for 7 min. The nested PCR was performed using the first PCR product as the template with the AP2 and Rev4 primers and the following PCR conditions: 5 cycles of 94°C for 25 s and 72°C for 3 min, 23 cycles of 94°C for 25 s and 67°C for 3 min and 67°C for 7 min.

The PCR product was electrophoresed through a 1% agarose gel, and a 1.5-kb product was confirmed. The amplified fragment was extracted using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), ligated to the pCR8/TOPO vector (Promega, Madison, WI, USA) with the TA-cloning method and transformed into *E. coli* strain DH5α. After 5′-end sequencing of the fragment using the M13 forward primer, a total 2,885 bp of sequence upstream of the *AgpS1* gene was obtained by high-fidelity PCR using genomic DNA as the template. After TA-cloning into the pCR8/TOPO vector, the insert was sequenced by primer walking with the primers shown in Figure S1A. Putative *cis*-element searches were performed with PLACE version 26.0 (http://www.dna.affrc.go.jp/PLACE/).

Construction of an AgpS1 promoter::GUS fusion vector

To construct an *AgpS1* promoter-β-glucuronidase (GUS) transformation vector, the 2,885 bp putative promoter sequence was integrated into the pGWB3 binary vector (Nakagawa et al. 2007) at a position upstream of the GUS reporter gene by an LA clonase reaction with the pCR8/TOPO vector. The vector was transformed into *Agrobacterium tumefacience* strain GV2260 by electroporation.

Transformation

The *AgpS1* promoter-GUS fusion gene (*PAgpS1::GUS*^{OX}) was transformed into tomato (*Solanum lycopersicum* cv., 'Micro-Tom') according to the method described in Sun et al. (2006), with minor modifications. The regenerated plants were evaluated for ploidy, and only diploid individuals were selected. The presence of the transgene and the copy number were confirmed by PCR using primers specific to the *GUS* and neomycin phosphotransferase II (*NPTII)* genes and Southern blotting, respectively. Finally, T_0 plants harbouring a single-copy transgene were screened. At the T_1 generation, homozygous lines were obtained and subjected to the GUS-staining experiments.

GUS staining and observation of tissue localisation

Seeds of wild-type and the transgenic tomato plants harbouring

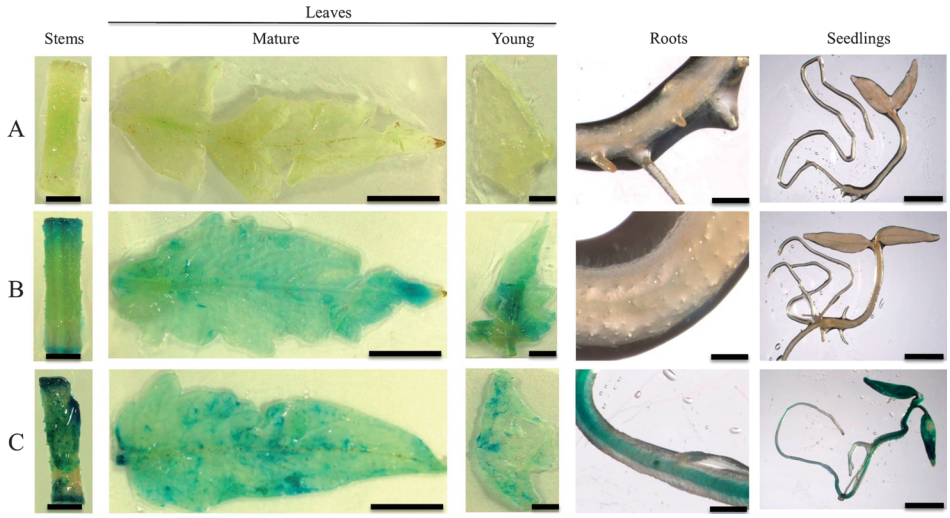

Figure 1. GUS staining patterns in stem, leaf, root and seedling. (A) Wild type, (B) *PAgpS1::GUS*^{OX} and (C) *P35S::GUS*^{OX}. *P35S::GUS*^{OX} plants are shown as a positive control of GUS staining. GUS staining was performed for 24 h with 0.5 mM X-Gluc. Bars=2 mm for stems and seedlings, 1 cm for mature leaves, 5 mm for young leaves and 400 μm for roots.

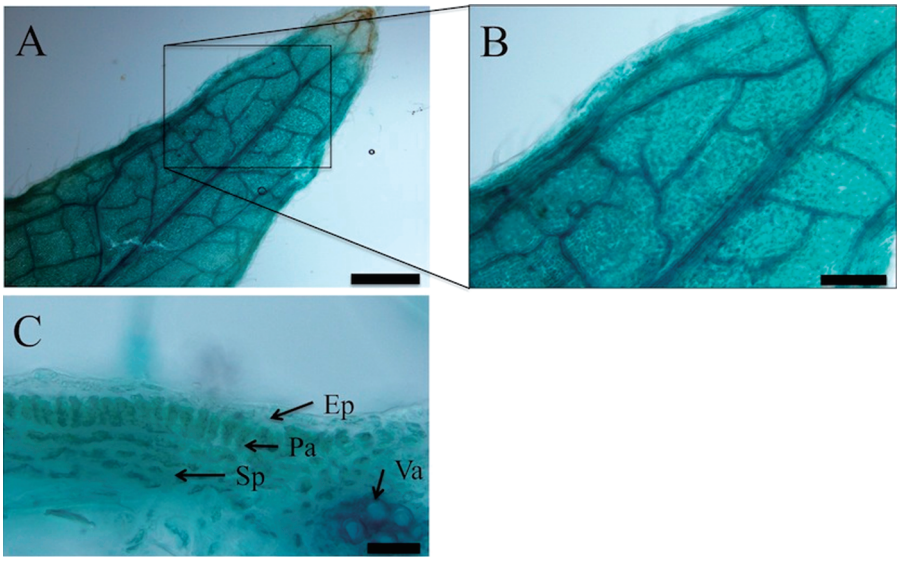

Figure 2. GUS staining of young *PAgpS1::GUS*^{OX} leaf. Young leaf surface (A) and (B) and cross-section of young leaf (C). The samples were observed using stereoscopic microscopy or light microscopy. Ep, Epidermis; Pa, Palisade layer; Sp, Spongy tissue; Va, Vascular tissue. Bars=600 μm (A), 200 μm (B) and 40 μm (C).

PAgpS1::GUS^{OX} and CaMV35S promoter-GUS fusion gene (*P35S::GUS*^{OX}) were sown on moist filter paper in a culture room at 25°C under a 16/8-h light/dark photoperiod condition. The seedlings were transplanted to rockwool pots at 1 week after germination. These plants were grown using hydroponic culture with a commercial nutrient solution (Otsuka A; Otsuka Chemical Co., Ltd., Osaka, Japan) adjusted to an electrical conductivity (EC) of 2.0 dS/m. The samples for GUS staining, including flower buds, flowers, fruits, fruit stalks, stems, leaves, roots and seedlings, were excised with a razor, immediately immersed in GUS staining solution (0.1 M $NaHPO_4$ [pH 7.0], 0.01 M EDTA, 0.005 M $K_3Fe(CN)_6$, 0.005 M $K_4Fe(CN)_6$, 0.1% Triton X-100 and 0.5 mg/l 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid cyclohexylammonium salt (X-Gluc) in a 12-well titre plate and incubated for 24 h at 37°C in the dark. After staining, the samples were incubated in 100% ethanol for 3 days for decolorization of the chlorophyll, changing the ethanol several times, and then stored in 50% glycerol solution. The tissue sections shown in Figures 2–4 and Figures S2 and S4 were prepared by hand sectioning with a razor. The GUS staining patterns were observed by stereoscopic- and optical microscopy.

Results

Isolation and sequence analysis of the AgpS1 promoter

A 2,885 bp fragment of the sequence upstream of the

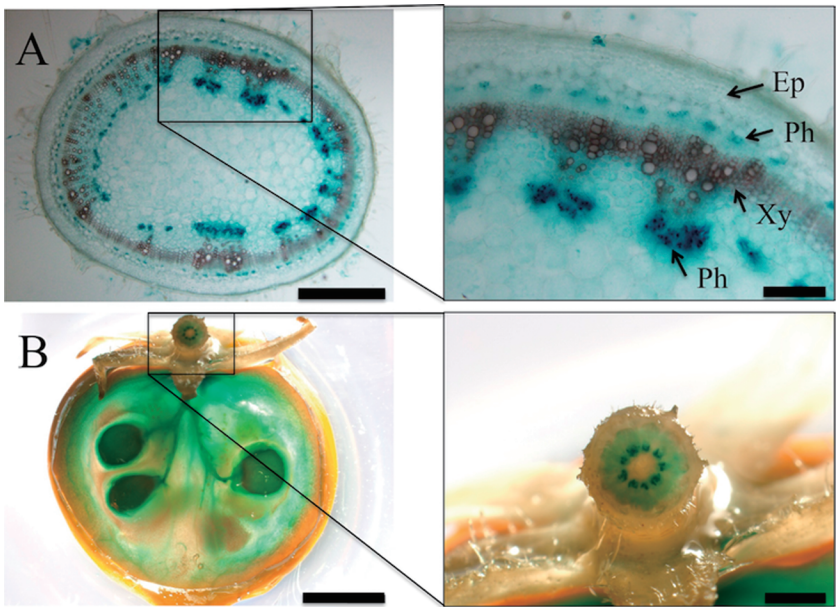

Figure 3. GUS staining pattern in *PAgpS1::GUS*^{OX} stem (A) and fruit stalk (B). GUS staining was performed for 24 h with 0.5 mM X-Gluc. Stem cross-sections were prepared by hand-sectioning with a razor. Stage of fruit in (B) is the red stage. Ep: epidermis. Ph: phloem. Xy: xylem. Bars = 600 μm (A, left), 200 μm (A, right), 4 mm (B, left) and 1 mm (B, right).

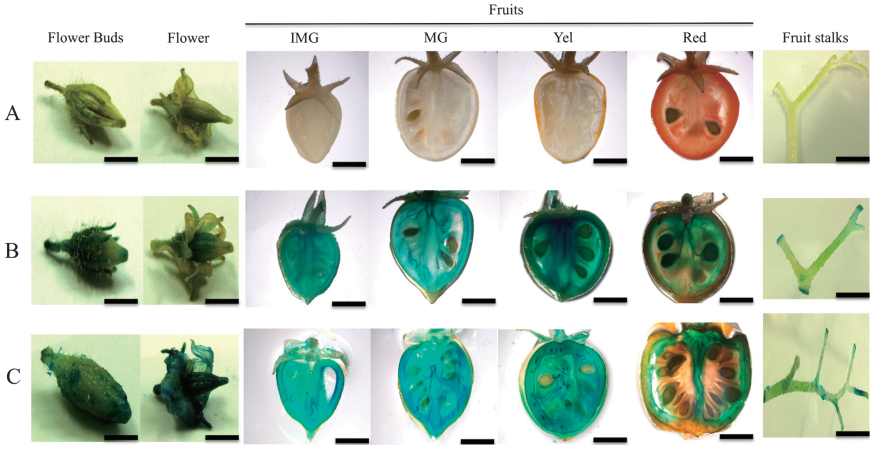

Figure 4. GUS staining patterns in flowers, fruit and fruit stalks. (A) Wild type, (B) *PAgpS1::GUS*^{OX} and (C) *P35S::GUS*^{OX}. Fruit stages were defined as immature (IMG), mature green (MG), yellow (Yel) and Red. GUS staining was performed for 24 h with 0.5 mM X-Gluc. *P35S::GUS*^{OX} plants are shown as a positive control of GUS staining. Bars = 2 mm for bud and at anthesis, 5 mm for fruit and 2 cm for fruit stalks.

AgpS1 gene was obtained by adaptor-specific PCR using the walking PCR library (GenBank accession no. AB824726) (Figure S1). Two TATA box-like elements at positions −88 to −94 bp (TATAAAA) and −114 to −120 bp (TATAAAG) were identified. The results of a *cis*-element search using PLACE indicated a number of putative *cis*-elements responsive to abiotic stress in the upstream region of the *AgpS1* gene (Table 1). For example, there are seven GT-1 motifs, which are found in the promoters of a soybean salt-induced CaM isoform gene, at −832 bp, −864 bp, −2,110 bp and −2,252 bp in the sense orientation and −1,123 bp, −1,197 bp and −1,446 bp in the antisense orientation. Additionally, eight MYB recognition sites, at −90 bp, −1,631 bp, −2,025 bp, −2,091 bp, and −2,599 bp in the sense orientation and −1,472 bp, −1,631 bp, and −2,761 bp in the antisense orientation, and four MYC recognition sites at −477 bp, −479 bp, −1,118 bp and −1,141 bp are also found in the promoters of *Arabidopsis* dehydration-responsive genes such as *rd22*. A number of *cis*-elements related to sugar repression such as sugar-repressive element (SRE) and pyrimidine box are also found in the *AgpS1* sequence at −499 bp, −1,259 bp, −1,449 bp, −1,856 bp and −2,814 bp in the sense orientation and −861 bp, −1,415 bp, −2,333 bp and −2,424 bp in the antisense orientation, which have been reported in the promoter sequence of cereal α-amylase genes. Apart from those *cis* elements, thirteen GATA motifs were found

Table 1. Putative *cis*-elements found in the *AgpS1* promoter region.

Putative *cis*-element	Position (bp) from ATG (+: sense, −:antisense)	Annotation
GACGTC	−438 (+, −)	C-box/RITA-1 binding domain site.
TAACARA	−10 (−),−2,600 (−)	Amylase box. Conserved sequence found in 5′ upstream region of alpha-amylase gene. C-box according to the nomenclature of ACGT elements. One of ACGT elements, factors groups 1, 2 and 3 has affinity for C-box and RITA-1 binding site. R=A/G.
CAATTATTA	−2,375 (−)	Consensus binding sequence for Arabidopsis homeodomain-leucine zipper protein, ATHB6 that is a target of the protein phosphatase ABI1 and regulates hormone responses.
ACCWWCC	−1,754 (+)	In *PAL1* promoter region, DCMYB1 bound to these sequences in vitro. Consensus of the putative "core" sequences of box-L-like sequences in carrot. W=A/T.
TGGACGG	−796 (+)	CMSRE (Carbohydrate Metabolite Signal Responsive Element 1) found in the promoter of sweet potato sporamin A gene.
ACACNNG	−480 (+), −2,070 (−)	DPBF-1 and 2 (Dc3 promoter-binding factor-1 and 2) binding core sequence which classified a novel member of bZIP transcription factors. Dc3 expression is normally embryo- specific, and also can be induced by ABA.
GANTTNC	−1,032 (+), −1,531 (−), −1,659 (+), −2,277 (−)	Consensus motif of the two enhancer elements, EE-1 and EE-2, both found in the promoter region of the *Chlamydomonas* Cah1 (encoding a periplasmic carbonic anhydrase). Binding site of Myb transcription factor LCR1. *n*=A/G/C/T.
GAAAAA	−832 (+), −864 (+), −1,123 (−), −1,197 (−), −1,446 (−), −2,110 (+), −2,252 (+)	GT-1 motif found in the promoter of soybean CaM isoform, SCaM-4, which plays a role in pathogen- and salt-induced SCaM-4 gene expression.
GATAAG	−369 (+)	I box; binding site of LeMYB1 which is a member of a novel class of myb-like proteins. LeMYBI acts as a transcriptional activator.
WAACCA	−90 (+), −1,472(−), −2,091 (+), −2,761 (−)	MYB recognition site found in the promoters of the dehydration-responsive gene *rd22* and many other genes in Arabidopsis. W=A/T.
YAACKG	−1,631 (−)	MYB recognition site found in the promoters of the dehydration-responsive gene *rd22* and many other genes in Arabidopsis. Y=C/T, K=G/T.
CNGTTR	−1,631 (+), −2,025 (+), −2,599 (+)	Binding site for at least two plant MYB proteins ATMYB1 and ATMYB2, both isolated from Arabidopsis. ATMYB2 is involved in regulation of genes that are responsive to water stress in Arabidopsis. *N*=A/G/C/T; R=A/G.
TAACAAA	−10 (−)	Central element of gibberellin (GA) response complex (GARC) in high-PI alpha-amylase gene in barley. GAMYB binds specifically to the TAACAAA box in vitro. GAMYB is the sole GA-regulated transcriptional factor required for transcriptional activation of the high-PI alpha-amylase. GARC consists of the pyrimidine boxes, which in RAmy1A are partially involved in sugar repression.
CCWACC	−1,757 (+)	Core of consensus maize P (MYB homolog) binding site; Maize P gene specifies red pigmentation of kernel pericarp. W=A/T.
GGATA	−1,246 (+), −1,414 (+), −1,826 (+), −2,332 (+), −2,423 (+), −2,457(+), −2,814 (−)	Core motif of MybSt1 (a potato MYB homolog) binding site.
CATGTG	−477 (+), −479 (−), −1,118 (+), −1,141 (+)	MYC recognition sequence is necessary for expression of *erd1* (early responsive to dehydration) and *rd22* genes in dehydrated Arabidopsis.
GNATATNC	−2,457 (+, −)	PHR1(phosphate starvation response 1)-binding sequence found in the upstream regions of phosphate starvation responsive genes from several plant species.
CCTTTT	−499 (+), −861 (−), −1,259 (+), −1,449 (+), −1,856 (+)	Pyrimidine box found in the promoter of barley alpha-amylase (Amy2/32b) and rice alpha-amylase (RAmy1A) genes, which are partially involved in sugar repression.
CATGCA	−886 (+), −2,117 (−)	"RY repeat" found in RY/G box (the complex containing the two RY repeats and the G-box) of *napA* gene in *Brassica napus*.
TTATCC	−1,246 (−), −2,332 (−)	Sugar-repressive element (SRE) found in 272 of the 1592 down-regulated genes after main stem decapitation in *Arabidopsis*.
GAGAC	−1,380 (+)	Core of sulfur-responsive element (SURE) found in the promoter of SULTR1, high-affinity sulfate transporter gene in Arabidopsis. SURE contains auxin response factor (ARF) binding sequence (GAGACA).

Table 1. Continued.

Putative *cis*-element	Position (bp) from ATG (+: sense, −:antisense)	Annotation
TATCCA	−1,415 (−), −2,333 (−), −2,424 (−), −2,814 (+)	TATCCA element found in alpha-amylase promoters of rice. Binding sites of OsMYBS1, OsMYBS2 and OsMYBS3 which mediate sugar and hormone regulation of alpha-amylase gene expression; a GATA motif as its antisense sequence is responsible for sugar repression.
TGACT	−164 (−), −249 (−), −743 (−), −1,714 (+)	SUSIBA2 binds to W-box element in barley *iso1* (encoding isoamylase1) promoter.
ANNNGATA	−309 (−), −366 (+), −594 (+), −649 (−), −701 (+), −994 (+), −1,242 (+), −1,486 (−), −1,632 (−), −1,763 (+), −2,328 (+) -2,669 (−), 2,758 (−)	GATA motif found in most of the light-regulated promoters, which is important for activity of those promoters and may be involved in phloem-specific gene expression.

at −366 (+), −594 (+), −701 (+), −994 (+), −1,242 (+),−1,763 (+) and −2,328 (+) in the sense orientation and −309 (−), −649 (−), −1,486 (−), −1,632 (−), −2,669 (−) and 2,758 (−) in the antisense orientation (Table 1).

Tissue/organ localisation of GUS activity driven by the AgpS1 promoter in transgenic plants

To clarify whether the obtained sequence has a functional promoter, we generated stable transgenic plants with the *AgpS1* promoter-GUS fusion construct (*PAgpS1::GUS*^{OX}). We obtained eleven diploid T_0 plants with a single-copy transgene and assessed the GUS activity in the early developing fruit by GUS-staining. Based on the results, three plants, No. 4, No. 38 and No. 109, were selected for further investigation (Figure S2). At the T_1 generation, multiple plants that were homozygous for the transgene were obtained by PCR and Southern blot analyses in each selected line. The GUS staining of the T_1 and T_2 plants showed that *AgpS1* was broadly expressed in both sink and source tissues/organs, including seedling, stem, flower, fruit stalk, fruit and root. In both young and mature leaves, GUS activity was observed in all tissues, except for the epidermal cuticle layer (Figures 1B and 2). In young leaf, GUS activity was also localised in the vascular tissue and palisade layer (Figure 2), though this pattern was not clear in mature leaf. There was almost no GUS activity in the seedlings of the *PAgpS1::GUS*^{OX} line, whereas a strong GUS staining was observed in the *P35S::GUS*^{OX} line, which was generated in a previous work and used as a positive control for GUS staining (Figure 1B,C). Only minimal expression was observed in the vascular tissues of the cotyledon and root of the *PAgpS1::GUS*^{OX} seedlings (Figures S3 and S4). In the stem and the fruit stalk of *PAgpS1::GUS*^{OX}, the GUS activity was very restricted to the vascular phloem tissue, showing a patchy staining pattern (Figure 3).

GUS activity was observed in the sepals, ovaries and receptacle tissues of the *PAgpS1::GUS*^{OX} floral bud and tended to be restricted to the base of the ovary and receptacle at flowering (Figure S5). In the fruit, the highest GUS activity was observed at the immature-green (IMG) stage in the *PAgpS1::GUS*^{OX} plants, whereas staining was found in the entire fruit with the *P35S::GUS*^{OX} construct (Figure 4). The GUS activity in the pericarp tissue of *PAgpS1::GUS*^{OX} decreased with fruit development and had almost disappeared by the Red stage, although the GUS activity tended to be maintained in the vascular tissues.

Discussion

In this study, 2,885 bp of genomic sequence upstream of *AgpS1* gene was isolated from tomato for the first time. The obtained sequence was proven to be the functional promoter using GUS staining. Sequence analyses revealed many predicted *cis*-elements that are responsive to abiotic stress, such as salt, dehydration and sugar (Table 1). In our preliminary experiment, *AgpS1* expression responded to proline at the transcriptional level (data not shown). However, a proline-responsive *cis*-element was not found in the promoter sequence. It was reported that a 3,178 bp promoter sequence of *AgpL1* encoding the AGPase large subunit has at least four possible *cis*-elements responsive to sugar (Li et al. 2002). Based on sequence comparison, there is little similarity between the promoters of *AgpS1* and *AgpL1*.

There are twelve conserved motifs relevant to the sugar repression in the *AgpS1* promoter sequence, including the pyrimidine-box found in cereal *α*-amylase genes (Table 1). However, our previous work reported that *AgpS1* was upregulated at the transcriptional level by salt stress but it did not respond to sugar (Yin et al. 2010). Although the *AgpL1* gene, which encodes a large subunit and is also highly expressed in early developing fruit, was upregulated by sugar (Li et al. 2002; Yin et al. 2010), there is a conserved motif related to sugar repression in its promoter sequence (Xing et al. 2005). Therefore, these motifs should not be involved in the expression of tomato AGPase genes. On the other hand, seven salt-induced GT-1 and thirteen GATA motifs were found in the *AgpS1* promoter (Table 1). It was reported that the GATA motif would be required for phloem-specific expression because it has been found in several phloem-specific gene promoters as a conserved motif (Yin et al. 1997).

This is consistent with our results of the promoter-GUS expression pattern observed in sink-type organs such as stem and fruit stalk (Figure 3). To clarify which element actually contributes to *AgpS1* expression, a promoter-deletion assay would be required.

GUS staining revealed that *AgpS1* was broadly expressed in both sink and source organs, although the tissue specificity was different among the organs. In the source leaf and immature green fruit, *AgpS1* was uniformly expressed (Figures 1B and 4B). A functional AGPase enzyme requires both of small and large subunits, and the large subunit genes *AgpL3* and *AgpL1* are the predominant transcripts in tomato source leaf and immature green fruit (Cheng et al. 1998; Park et al. 1998). In transgenic tobacco and tomato plants, tissue localisation revealed that *AgpL3* was expressed throughout the leaf, whereas *AgpL1* was restricted to the inner pericarp and columella of the early developing fruit (Xing et al. 2005). Our results on *AgpS1* for source leaf were consistent with previous reports but not with regard to the early developing fruit (Figures 1B, 2 and 4B).

We found that expression was restricted to the vascular tissue in sink-type organs, such as stem, fruit stalk, cotyledon and root (Figures 3, S3 and S4). It was reported that AGPase expression tended to be regulated at the transcriptional level in sink organs (Anderson et al. 1991), suggesting that the AGPase mainly functions around the vascular tissue in sink organs. The present results are consistent with the previous observations in rice leaf sheath tissue, whereby starch granules tended to accumulate around the vascular bundles (Matsukura et al. 2000). A patchy staining pattern was observed in phloem tissue of the stem and fruit stalk. Such a patchy pattern is characteristic of phloem companion cells (Matsukura et al. 2000), suggesting that *AgpS1* is expressed in the companion cells in the stem and fruit stalks. Although the regulation of *AgpS1* in companion cells remains unknown, it is likely that components transported via phloem are involved in this regulation.

As described above, *AgpS1* responded to proline treatment (data not shown), which is known as a major compatible solute in plants. Indeed, a phloem-specific proline transporter (*AtProT1*) was reported in *Arabidopsis*, and its role in long-distance phloem transport via the vascular system was suggested (Grallath et al. 2005). Considering the phloem-specific expression of the *AgpS1* gene, it is likely that proline transported via phloem is involved in the regulation of *AgpS1* expression. As *AgpS1* exhibits different expression pattern by the organs and developmental stages, it would be interesting to investigate a correlation between the expression pattern and proline distribution in tomato plants.

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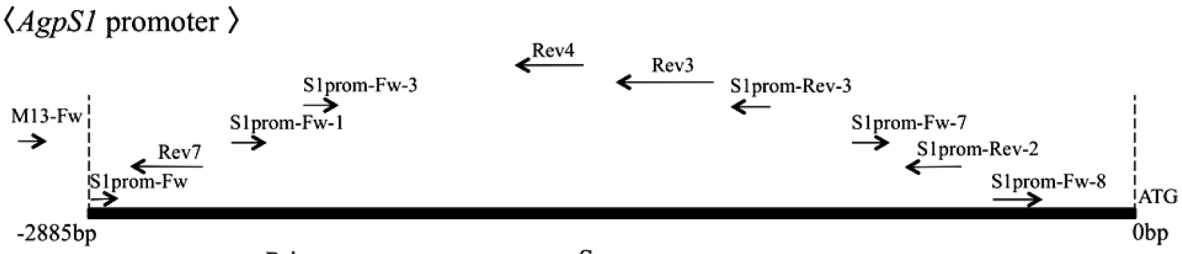
A



Primer	Sequence
M13-Fw	5'-GTAAAACGACGACGGCCAG-3'
S1Prom-Fw	5'-CCTCTGCCAAAACAGCTCAG -3'
S1Prom-Fw-1	5'-GTTGGAGGATATTCTCTCCTTT -3'
S1Prom-Fw-3	5'-GTCACTTGACCAAAGATCAAC-3'
S1Prom-Fw-7	5'-TGACGGGATTGATGTTCACTG-3'
S1Prom-Fw-8	5'-CTAACTCTCGTGCAAACAAGTG-3'
S1Prom-Rev-2	5'-CTGGTACTTGTAGACATGACAA-3'
S1Prom-Rev-3	5'-GAGCAAACACAACTTTCTATC-3'
Rev-3	5'-GTCCATCCCTGGCACACTGCGGTGCAAG-3'
Rev-4	5'-CCACCTGAAGGCCCCGGAATACCTCC-3'
Rev-7	5'-CAGATGTCCACGGAAATTAGCCACAC-3'

Figure S1. List of primers for isolation and sequencing of the *AgpS1* promoter region (A) and the isolated sequence (B).
Fw, forward primer; Rev, Reverse primer."ATG" indicates the start codon of the *AgpS1* gene.

B

CCTCTGCCAAAACAGCTCAGAATGTTGAAGAGGTGGGGGATTCTTCCATCCGCTACTTTCCTGACCTTTAATATCCATCCATGGACATCT

TTCTCTAACTAAACTTATAAATTGGTTGTTTATCTGGTTTCTTGTTTCTGCTGCTTTTCAGGCTTTTATCAGAACAGCCTCAACAATTTATA

AGAAAATACAGGATGGAGTGTTTGATGTATCAAATGAGGTATGTCACGCCCTGACCATTTTTATATATCTATAGTCTTTTAGTGTGGCTAAT

TTCCGTGGACATCTGTTAATATGAAAATGGCGATCGTCTAGAAAGACATAATTGACATCTCATGAAGTAAAGAGTTATGAAGAAGGTTTT

TTTTGCTTGTTCTAAACCTGTTTTAATTTTGTGTTCTTGATTGAAATTAAATAAAAAAGTTGGAGGATATTCTCTCCTTTGAAAACATATAT

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TGTATTTGCAAATGTCTAGCTGTATTATTTGCAGATAAATGCTGTTATAGCTTCTGAGATGCCAGTTTCCGTGATGAATAGTCTTGCAAAAT

GTAACATCCAAATTGGGAGTAGATGTTTAAGCAGTTCCTAATTGTATCAGTCTTCTCATTTTCTGGGATCTTTAATTCATCTACTTTGACCT

ATAAAAATAATGTGTGAATTCAGTGTCCTTTTGAGAGCTAGATACCTAGAACTCACGGATATGGAACATGCTGTCTTAGTGTGGGAAGGC

AACATTAATTTGGAGGAAGTTAAGAAGATATTATTCCAACCATCCAAAGAAATACCGATCCATCAATCTGCATTCTGCTGACTTGTGAGTT

CATACCCCAATCTGGGACCAATAGTTGGGCATATTGTTAGCAGAGTTCCCTGTTTAATGTAGTATCATTTCAGTTGAGTAGTGATAGAATA

GCATTGATGAATGTCCTAATCATTGTTTTGAGAACCAAGAAAACAGAAACTTACGTTGCAAGTATTTCAGTAAGTACTTGTAAATCTCAG

TGATAATTGATTATCAACATGTATAATTATCATTTACGTAATAGTAGATGGTTTCCGAAACTAATGCTTGCCCTTTTTTCATTTGCAGTCCTA

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TCAACTCTCATAGGGAAAGTGACAGCATAGAATAAAGTATAATATCAATTCTGGCAATTTCGAATTATTCCACATCTCTTTTGTCATGTCTA

CAAGTACCAGATACCAATTATTTAAATACAAAAAAATCTTGATTAAACAATTCATTTTCTCACTCTTAATCACATTTAATAATGCACGGCTC

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GCATATAAGAATATCTTTTAATCTTGTTTTTAAAAAACAACGCAGCTCAAATAAAATAAAATAAAGCTAAAATACAAAAGTCATATTTCAA

TCCCCAAAATACCCTCAATCGCAATAAATATTTCAATCCCCAAAATACCCTCAATCACAAGAAGTGTGTACCAGTCACACCTATGGTCCT

CTCGTAAATTCCCACAAAACTAAGTCTATAAAGTTACCCTTGATATCAGTATTATAAAACCAAAAATCTCAGCTGTAATTAAGTGCAATCA

CACTCTACCACACACTCTCTATAGTATCTATAGTTGAGAGCAAGCTTTGTTAACA

Figure S1. List of primers for isolation and sequencing of the *AgpS1* promoter region (A) and the isolated sequence (B) (continued)
Predicted TATA boxes are indicated with yellow color.

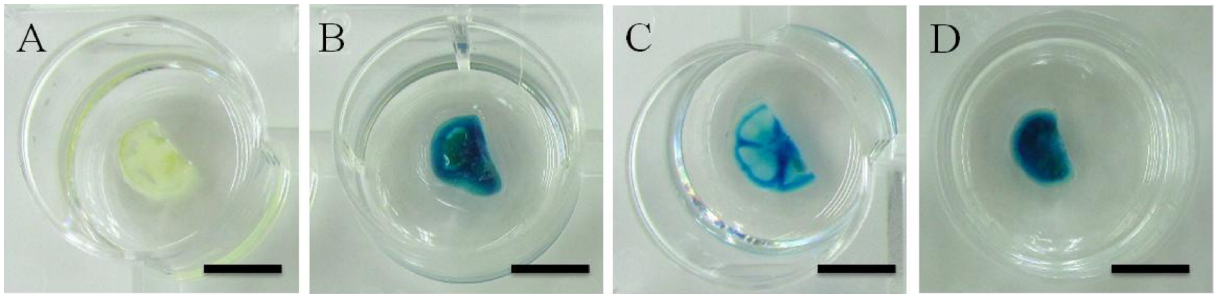

Figure S2. GUS staining of the *PAgpS1::GUS*^{OX} T_0 generation in immature fruits.

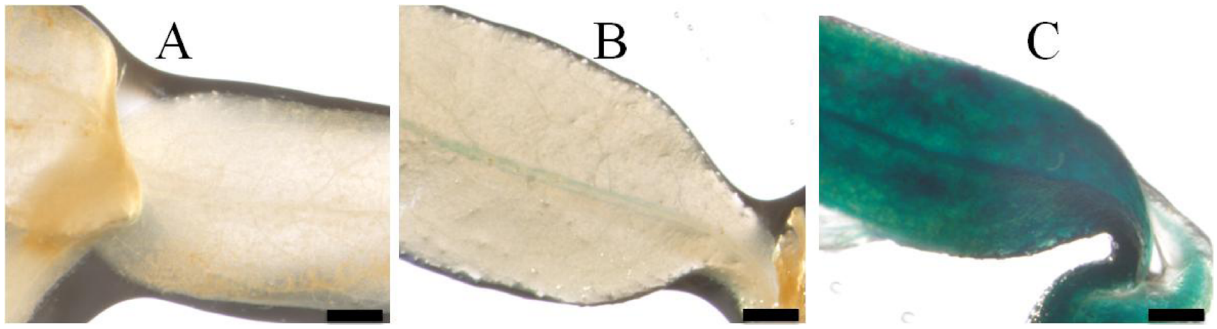

Figure S3. GUS staining pattern in seedling cotyledons.

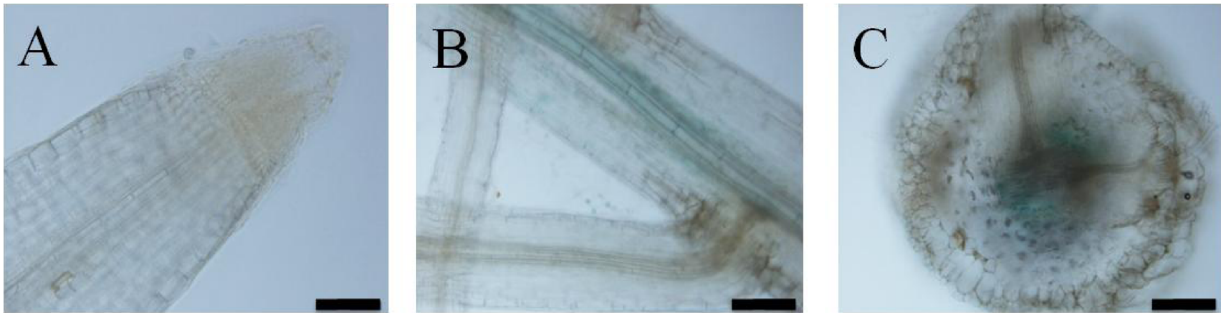

Figure S4. GUS staining pattern in *PAgpS1::GUS*^{OX} seedling roots.

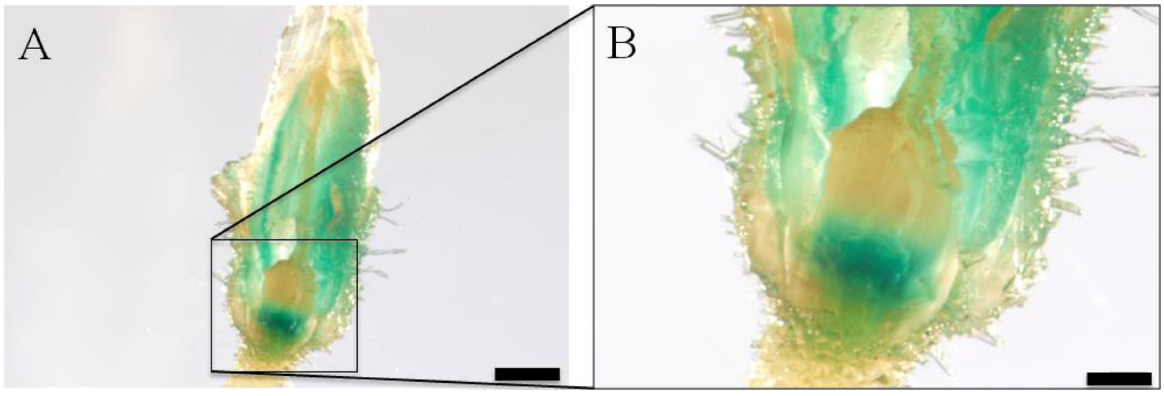

Figure S5. GUS staining pattern in *PAgpS1::GUS*^{OX} flower.