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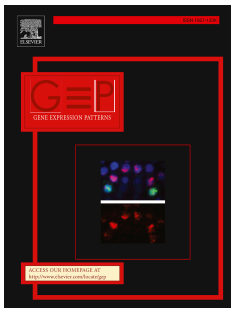

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Arabidopsis thaliana sucrose phosphate synthase (*sps*) genes are expressed differentially in organs and tissues, and their transcription is regulated by osmotic stress

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Short title:
Arabidopsis *sps* genes

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ABSTRACT

Sucrose is synthesized from UDP-Glc and Fru-6-phosphate via the activity of sucrose-phosphate synthase (SPS) enzymes, which produce Suc-6-phosphate. Suc-6-phosphate is rapidly dephosphorylated by phosphatases to produce Suc and inorganic phosphate. Arabidopsis has four *sps* genes encoding SPS enzymes. Of these enzymes, AtSPS1F and AtSPS2F have been grouped with other dicotyledonous SPS enzymes, while AtSPS3F and AtSPS4F are included in groups with both dicotyledonous and monocotyledonous SPS enzymes. In this work, we generated *Arabidopsis thaliana* transformants containing the promoter region of each *sps* gene fused to *gfp::uidA* reporter genes. A detailed characterization of expression conferred by the *sps* promoters in organs and tissues was performed. We observed expression of *AtSPS1F*, *AtSPS2F* and *AtSPS3F* in the columella roots of the plants that support sucrose synthesis. Hence, these findings support the idea that sucrose synthesis occurs in the columella cells, and suggests that sucrose has a role in this tissue. In addition, the expression of *AtSPS4F* was identified in embryos and suggests its participation in this developmental stage. Quantitative transcriptional analysis of *A. thaliana* plants grown in media with different osmotic potential showed that *AtSPS2F* and *AtSPS4F* respond to osmotic stress.

Key words: sucrose, genes, transcription, osmotic stress.

INTRODUCTION

Plants are photosynthetic organisms that use light energy, carbon dioxide from the atmosphere, and water for carbohydrate synthesis in the chloroplast. They transport cytoplasmic triose phosphates, which permits the synthesis of sucrose in several reactions. Further, sucrose is synthesized from UDP-Glc and Fru-6-phosphate via the activity of sucrose-phosphate synthase enzyme (SPS; EC 2.4.1.14), which produce Suc-6-phosphate. Suc-6-phosphate is rapidly dephosphorylated by a phosphatase (SPP; EC 3.1.3.24) to produce Suc and inorganic phosphate (Pi), making the reaction practically irreversible (Huber and Huber, 1996).

In most plants, sucrose is the main sugar transported from the site of synthesis to other organs and tissues of the plant for utilization. Sucrose is transported in phloem tissue and is discharged from it by specific membrane transport proteins. Once in this tissue, sucrose and other organic compounds are mobilized by mass flow. Sucrose is cleaved in tissues by the enzyme sucrose synthase (Sus; EC 2.4.1.13) which produces UDP-Glc and Fru. Conversely, sucrose can be hydrolysed by invertase (INV; EC 3.2.1.26) to yield Glc and Fru. Both processes allow monosaccharides to be used for energy production and the synthesis of cellulose, starch, fructans, protein and antioxidant compounds (Ruan, 2014; Jiang et al., 2015). Prior to sequencing of plant genomes, the SPS enzyme was thought to be encoded by one gene in each species. SPS cDNA has been reported for maize (Worrell *et al*., 1991), spinach (Klein et al., 1993; Sonnewald et al., 1993), banana (do Nascimento et al., 1997), sugar beet (Hesse et al., 1995), *Citrus unshiu* (Komatsu et al., 1996), fava bean (Heim et al., 1996), *Craterostigma plantagineum* (Ingram et al., 1997), sugarcane (Sugiharto et al., 1997), and kiwi fruit (Langenkämper et al., 1998); additionally, a genomic sequence was reported in rice (*OsSPS1*) (Valdez-Alarcón et al., 1996). However, recent work has shown that a family of *sps* genes exists in different plant species (Jiang et al., 2015). Langenkämper et al. (2002) classified the SPS enzymes into three groups, designated A, B and C. In contrast, Castleden et al. (2004) classified SPS

enzymes into four groups (A, B, C and D), and Lutfylla et al. (2007) classified six groups (1a, 1b, 2d, 2m, 3 and 4). These groups were classified based amino acid sequence similarity within the glucosyl transferase enzyme domain, among which 1a and 1b correspond to bacterial SPS. Arabidopsis has 4 *sps* genes encoding SPS enzymes. AtSPS1F (AtSPS1A) and AtSPS2F (AtSPS2A) have been grouped with other dicotyledonous SPS enzymes in the 2d group. Meanwhile, AtSPS3F (AtSPSB) and AtSPS4F (AtSPSC) are included in groups 3 and 4, respectively and contains both dicotyledonous and monocotyledonous SPS enzymes (Lutfylla et al., 2007).

The route of sucrose synthesis is highly regulated at the transcriptional level for genes encoding the SPS enzymes, by covalent modifications which result in enzyme activation or inactivation and via the allosteric effects of some substrates of this sugar associated with metabolic pathways (Huber and Huber, 1996). Allosteric regulation occurs in some SPS enzymes such as spinach and maize, where the Glc-6-P, a precursor in the synthesis pathway, positively regulates its activity, whereas inorganic phosphate (Pi), one of the final products, negatively regulates the activity (Amir and Preiss, 1982; Doehlert and Huber, 1983; Kalt-Torres et al., 1987). However, allosteric regulation is not a general feature of SPS enzymes because it has been reported that leaf soybean and wheat germ SPS are only inhibited slightly by Pi and are not affected by Glc-6-P (Nielsen and Huber, 1989; Salerno and Pontis, 1978). Additionally, AtSPS3F has been reported to be is allosterically regulated in *A. thaliana* (Volkert et al., 2014).

Light regulates various processes in plants, such as germination, flowering and photomorphogenesis (Bou-Torrent et al., 2008). This factor is important in the regulation of certain SPS enzymes, as phosphorylation or dephosphorylation determines protein activity (Stitt et al., 1988). In spinach (*Spinacia oleracea*), the RMRRISSVE sequence has been defined, in which serine 158 (bold) can be phosphorylated in the dark, inactivating the enzyme, and dephosphorylated in the presence of light, activating the enzyme (McMichael et al., 1993). An amino acid

sequence of an SPS for maize has been described and is similar to that of spinach. Moreover, the maize SPS has a serine at position 162, which can be phosphorylated in the dark, inactivating the enzyme (McMichael et al., 1995). Other SPS enzymes have been described as potential sites of regulation by light-dark, as in rice (Huber and Huber, 1996) and in several dicotyledonous plants, including *A. thaliana* (Lutfylla et al., 2007).

During drought and high salinity, water availability for plants is severely limited. Due to osmotic effects derived from molecules that interact with water, its free energy status (water potential) is decreased, making it more difficult to capture (Xiong and Zhu, 2002). Plant responses to osmotic stress are diverse and result in growth changes, adjustments in ion transport and changes in carbon metabolism. These changes can occur in response to the osmotic effect of primary signals or secondary signals such as abscisic acid and ethylene hormones (Fujita et al., 2011). Covalent regulation of SPS enzymes can also occur by osmotic stress. In spinach, it has been delimited by the RMRRGV**S**C sequence, with a serine at position 424. Moreover, serine 424 is phosphorylated when the plant is subjected to osmotic stress and the enzyme is activated, perhaps antagonizing the inhibitory effect of the phosphorylation of serine 158 to permit the synthesis of sucrose (Winter and Huber, 2000). In other species of dicotyledonous plants and monocots, regulation by water stress and the presence of similar amino acid sequences which may contain putative serine phosphorylation sites have been reported. In *A. thaliana*, AtSPS1F, AtSPS2F and AtSPS4F, this regulatory sequence is present; however, in AtSPS3F, it is absent (Lutfylla et al., 2007).

The transcriptional regulation of *sps* genes is complex, and it is increasingly important considering that there are several genes encoding SPS enzymes in each plant species. The promoter regions of different *sps* genes confer expression that differ totally or partially among the tissues and organs of plants. Moreover, in some species, these aspects have been addressed by qPCR used to quantify the number of each *sps* gene transcript present. Among the seven *sps* maize genes,

ZmSPS1F and *ZmSPS2F* show their highest expression in the leaves (34 and 29%, respectively), and (*ZmSPS6*) shows expression only in the inflorescence, with the other five genes showing expression distributed among different organs (Lutfylla et al., 2007). Among the five *sps* genes in rice, *OsSPS1* shows greater expression, particularly in the leaves, and *OsSPS11* demonstrates marked expression in the roots (Okamura et al., 2011). In *A. thaliana*, patterns of expression conferred by the promoter region of *sps* genes have been reported in transgenic plants. Moreover, the study showed that *AtSPS2F* is expressed only in the roots, whereas the other three genes (*AtSPS1F*, *AtSPS3F* and *AtSPS4F*) are expressed in leaves, stems and flowers (Volkert et al., 2014).

Genes encoding SPS enzymes can be regulated at the transcriptional level during the development of plants. For example, an increase occurs in the *sps* transcripts during the maturation of spinach leaves (Klein et al., 1993), and fruits in mandarin (Komatsu et al., 1996), banana (Oliveira et al., 1997) and kiwi (Langenkämper et al., 1998). Additionally, there is increased transcription in *OsSPS1* in the maturation of rice leaves and in the seed scutellum during germination (Chávez-Bárcenas et al., 2000; Martínez-Trujillo et al., 2004).

Osmotic stress may transcriptionally regulate the expression of the *sps* genes. In maize, it was determined by qPCR that water stress induces *ZmSPS3F*, represses *ZmSPS1F* and *ZmSPS4F*, and the other four genes do not have a significant change in expression (Lutfylla et al., 2007). In *Craterostigma plantagineum* Hochst, a plant with tolerance to severe desiccation, there is an increase in *sps* transcription during plant dehydration (Ingram et al., 1997). For some *sps* genes, light has an effect on their transcriptional regulation. To illustrate, there is an increase in the transcription *of OsSPS1* in rice leaves in the presence of light (Chávez-Bárcenas et al., 2000). Yonekura et al. (2013) determined that during the process of circadian rhythm there is a different pattern in the transcriptional expression of the five *sps* genes of rice measured by qPCR.

Complexities that exist in the regulatory processes that lead to sucrose synthesis make it necessary to delve deeper into certain aspects of these processes. Thus, this work contributes to the following: a) the generation of transformants containing the promoter region of each *sps* gene fused to the *uid*A*::gfp* reporter genes, b) a detailed characterization of the expression conferred by *sps* promoters in organs and tissues, complementing previously reports, c) the determination of the *sps* transcription expression levels, and d) the analysis of the effect of osmotic stress on the transcriptional expression levels of the four *sps* genes.

MATERIALS AND METHODS

Transgenic lines

Abi4::uidA (Söderman et al., 2000).
*AtSPS1F::uid*A*-gfp* (this work).
*AtSPS2F::uid*A*-gfp* (this work).
*AtSPS3F::uid*A*-gfp* (this work).
*AtSPS4F::uid*A*-gfp* (this work).

Arabidopsis thaliana genomic DNA extraction and amplification of the promoter regions

The sequences of *AtSPS1F* (At5g20280), *AtSPS2F* (At5g11110), *AtSPS3F* (At1g04920), and *AtSPS4F* (At4g10120) 5' regulatory regions were obtained from the TAIR database (http://www.arabidopsis.org/). The oligonucleotides were designed with the Oligo Analyzer 3.0. Integrated DNA Technologies (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/) program.

The DNA of the wild-type *A. thaliana* ecotype Col-0 was isolated using the DNAeasy Plant Mini Kit (QIAGEN), following the manufacturer's instructions. The resulting DNA was used to amplify 1,500 bp of the *AtSPS1F*, *AtSPS2F*, *AtSPS3F* and *AtSPS4F* promoter regions, using the following oligonucleotides:

SPS1F 5'-GAGTTCTACATGAAGAAGATCCAGAAGAAGAAG-3'
SPS1R 5'-CTGGTGGGACGATCAAAGATCGA-3'

SPS2F 5'-AGCAGATGCATTTGTGGTAATCACATT-3'
SPS2R 5'-TGGTGTTCGCTCTCCCGAACT-3'
SPS3F 5'-ACCTATTTGCATCCGTGCTAGTTAGAG-3'
SPS3R 5'-TCTGAGTTTGTCCTCTGTTTCTCGAG-3'
SPS4F 5'-GAATCGTAGAAAGAGTCGAATGGTCAC-3'
SPS4R 5'-CTCTCTGATTGTTGCACTGAAAACTCC-3'

The Supermix High Fidelity® PCR kit (Invitrogen) was used following the manufacturer's instructions. The amplification conditions were as follows: 94℃ for 5 min, 30 cycles of 94℃ for 1 min, 58℃ for 30 s, 68℃ for 90 s, followed by a final extension at 68℃ for 10 min.

Construction of vectors to analyse the expression conferred by the *AtSPS1F*, *AtSPS2F*, *AtSPS3F*, and *AtSPS4F* promoter regions

The 1,500 bp PCR fragment, corresponding to the *AtSPS1F*, *AtSPS2F*, *AtSPS3F*, and *AtSPS4F* promoters, were cloned into the pDONR221® (Invitrogen) vector. The recombination reaction was performed using the BP Clonase Enzyme Mix Kit® (Invitrogen), forming the *attL1* and *attL2* recombination sites. The sequences and orientation of the cloned fragments were verified by the method of Sanger et al. (1977). The resultant vectors were recombined with the pKGWFS7 binary vector (Ghent University, Belgium) containing the *attR1* and *attR2* recombination sites; this vector harbours a translational fusion of the *uid*A and *gfp* reporter genes, allowing both to be used to analyse the expression conferred by the *AtSPS1F*, *AtSPS2F*, *AtSPS3F* and *AtSPS4F* promoter regions. Recombination was performed using the LR Clonase II enzyme Mix® Kit (Invitrogen).

***Agrobacterium tumefaciens* and *Arabidopsis thaliana* genetic transformations**

Recombined binary vectors were used to transform the *Agrobacterium tumefaciens* strain pGV2260 (McBride and Summerfelt, 1990) by electroporation at 1,800 V, using the Eppendorf electroporator 2510. Transformed colonies were selected in

LB medium (Luria) with carbenicillin (100 µg/ml^{-1}), rifampicin (50 µg/ml^{-1}), spectinomycin (100 µg/ml^{-1}) and streptomycin (300 µl/ml^{-1}).

The *A. tumefaciens* strain pGV2260 containing the recombinant binary vector was grown to an optical density of 0.6 (600 nm) and centrifuged for 5 min at 6,000 rpm; the pellet was resuspended in infiltration medium: 0.5x MS (Murashige and Skoog, 1962), 5% sucrose (Bioxon) and 0.05% Silwett L-77. The bacterial suspension was applied to 2- to 10-mm *A. thaliana* Col-0 inflorescences; the plants were placed in the dark for 12 h and then transferred to light conditions. To collect the seeds, the plants were allowed to produce mature siliques. Plant selection was made in 0.2x MS medium, pH 5.7, supplemented with 0.6% sucrose as a carbon source and 1% plant agar (Phytotechnology Laboratories A111), with kanamycin (60 µg/ml^{-1}).

Quantitation of the proline concentration in *A. thaliana* under osmotic stress

Proline was measured as described by Bates et al. (1973): 0.3 g of the frozen plant material was homogenized in 3% aqueous sulphosalicylic acid (0.02 g/ml^{-1}), and the residue was removed by centrifugation at 12,000 g for 10 min. One ml of the homogenized tissue reacted with 1 ml of acid-ninhydrin and 1 ml of glacial acetic acid in a test tube for 1 h at 100℃, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 1 ml of toluene. The chromophore-containing toluene was warmed to room temperature, and its optical density was measured at 520 nm in a Thermo Spectromic model Genesis 10 UV. The amount of proline was determined from a standard curve using D-Proline in the range of 5-60 µg/ml^{-1}.

Expression analysis by real-time quantitative PCR (RT-qPCR) in transformed *A. thaliana* lines under osmotic stress

Total RNA from Col-0 and transformed *A. thaliana* lines were isolated using Trizol® (Invitrogen). cDNA synthesis was performed using reverse transcriptase H (Thermo Fisher Scientific) and oligo dT. The following oligonucleotides were designed to amplify a 161 bp *AtSPS1F* (At5g2BF,

5'AGCAAGTTGGTGTTGGGAAG3', At5g2BR 5'TTGTCTCAAAAGCTGCTCCA3'); 245 bp *AtSPS2F* (At5g1AF, 5'TGTTTCAACCTTGGCACAAA3', At5g1AR 5'GTGGTTTCAACCCTCCTGAA3'); 215 bp AtSPS3F (At1gBF, 5'TGGATCCACACGATCAAGAA3', At1gBR 5'ATCGTCTTGAGCAGCCACTT3'); and 181 bp *AtSPS4F* (At4gAF, 5'GGAGCGTTAAATGTGCCAAT3'; At4gAR, 5'GTGTGCTTGTCACCACCATC3'). A 226-bp fragment of actin was used as the internal reference, using the oligonucleotides FACTINA, 5'TGCCAATCTACGAGGGTTTC3', and RACTINA 5'TTCCGATGGAAGAGCTGGT3'.

Growth conditions of different *A. thaliana* lines

The seeds of the different *A. thaliana* lines were surface disinfected by washing with 96% ethanol (v/v in water) for 7 min and then with 20% sodium hypochlorite (v/v in water) for another 7 min. Subsequently, the seeds were rinsed five times with sterile deionized water, resuspended in 1 ml and maintained at 4℃ in the dark for 48 h to promote and synchronize germination. Disinfected seeds were placed in Petri dishes containing 0.2X MS medium, pH 7.0, for plant growth, supplemented with 0.6% sucrose and 1% plant agar. For the selection of genetically transformed plants, kanamycin (60 $\mu g/ml^{-1}$) was added to the medium. Ten days after germination, the plants were transferred to pots with a substrate composed of peat moss, perlite, and vermiculite at a 3:1:1 ratio at field capacity of water. Both Petri dishes and pots were incubated in growth chambers (Percival Scientific AR-95L), under the following conditions: 24℃, 100 $\mu mol/m^2s^{-1}$ light intensity, 80% relative humidity, and a 16-8 h light-dark photoperiod.

For analysis by osmotic stress, the disinfected seeds were placed in Petri dishes containing 0.2x MS medium, pH 7.0, for plant growth, supplemented with 0.6% sucrose and 1% plant agar, and then mannitol (0, -0.3, and -0.6 Mpa) was added to the medium. The Petri dishes were incubated for ten days in growth chambers (Percival Scientific AR-95L), under the following conditions: 24ºC, 100 $\mu mol/m^2s^{-1}$ light intensity, 80% relative humidity, and a 16-8 h light-dark photoperiod.

Expression analysis of the *uidA* and *gfp* reporter genes

The transgenic plants with the *uid*A reporter gene (Jefferson et al., 1987) were stained with 0.1% 5-bromo-4-cloro-3-indolil D-glucuronide (x-gluc) in phosphate buffer (NaH_2PO_4 and Na_2HPO_4, 0.1 M, pH 7), with 4 mM potassium ferrocyanide and potassium ferricyanide for 12 h at 37℃. A blue compound was generated for conversion of the x-gluc substrate by the activity of the beta-glucuronidase enzyme encoded by *uid*A. The plants were clarified and fixed according to Malamy and Benfey (1997).

The expression of the *GFP* reporter gene was determined by detecting the green fluorescence emitted by the GFP protein (Haseloff and Amos, 1995). To analyse the expression pattern, seedlings were incubated with propidium iodide (1 μg ml^{-1}), which emits red fluorescence. The detection of GFP and propidium iodide fluorescence was performed using an Olympus Fluo-View FV1000-PME microscope. To measure the fluorescence of the GFP protein and propidium iodide, excitation wavelengths of 488 nm and 493 nm were used, respectively. Photographs of the GFP protein and propidium iodide were captured at 509 nm and 535 nm wavelengths, respectively.

RESULTS

Although the expression patterns of the four *sps* genes in *A. thaliana* has been reported, we provide a detailed characterization of the transcriptional expression of *sps* genes using the transgenic lines *AtSPS1F::uid*A-*gfp, AtSPS2F::uid*A-*gfp*, *AtSPS3F::uid*A-*gfp* and *AtSPS4F::uid*A-*gfp*. Steps for generation of the genetic constructs are shown in Fig. S1, and the selection of transformed plants is shown in Fig. S2.

For each *sps* gene, expression conferred in tissues and organs was evaluated in 10 transformed lines. The expression found in vegetative organs by analysing the histochemical changes produced by the enzyme encoded by the *uidA* reporter gene is shown in Fig. 1: overlaps in the expression in some organs were observed but also important differences, mainly that *AtSPS3F* was expressed in the roots, *AtSPS4F* was expressed in the apical stem, while *AtSPS1F* and *AtSPS2F* were expressed in the leaves and roots. By analysing GFP fluorescence, the detected expression in the roots was delimited to the stele and columella (Fig. 2). In plants grown for 3-4 weeks, *uid*A expression was analysed in flowers, the four *sps* genes were expressed in this organ, and *AtSPS4F* was expressed in developing embryos (Fig. 3). (Table 1).

Transcript levels of each *sps* gene was determined in *A. thaliana* Col-0 plants grown under *in vitro* conditions. Although *AtSPS2F* had a higher expression level than the other three *sps* genes, the increase was approximately 25% (Fig. 4).

To determine the effect of the osmotic stress on the accumulation of the osmoprotector proline and root growth, Col-0 and transgenic SPS cell lines were grown in the media with mannitol. We found that proline was duplicated and triplicated in plants grown in media with osmotic potential -0.3 and -0.6 MPa, respectively. Conversely, primary root growth was inhibited (Fig. 5). Transcript levels measured by qPCR for the Col-0 line showed that osmotic stress acts differentially on the induction of *sps* expression genes and show that *AtSPS2F* expression is increased to more than four times its basal level (Fig. 6). Moreover, the analysis among the four SPS transgenic lines was similar (data not shown).

We established a system for promoting osmotic stress and wanted to test whether this could induce expression of the abscisic acid response marker *abi4::uidA*. However, we found no difference in histochemical marker expression of *abi4::uidA* in *A. thaliana* grown in osmotic stress inducing media (Fig. 7).

DISCUSSION

The regulation of sucrose synthesis involves different steps from transcription to allosteric regulation (Huber and Huber, 1996). Thus, it is necessary to understand this process in greater detail. In this paper, the expression conferred by the promoters of the four *A. thaliana sps* genes in different tissues and organs was analysed. Moreover, the use of two genetic markers (*uidA* and *gfp*) allowed us to detail their expression, and our results were consistent with previous findings. Additionally, it was determined that these genes respond differently to osmotic stress via transcriptional expression.

In the evolutionary process, one of the major sources that generates diversity is gene duplication that can result in molecular mutations. Genetic mutations can change the sequence of the coding and regulatory regions of proteins forming families of genes that differ both in intensity, time and place of transcription and in the activity or function of the encoded protein (Panchy et al., 2016). Transcriptional expression conferred by the promoters of the *A. thaliana sps* genes in vegetative structures found in this work show some differences with a previous reported by Volkert et al. (2014) (Table 1). Instead of indicating contradictions, our results complement those previously reported. Some factors may explain variations in our results compared to others: i) use of the *gfp* marker in the roots delimits accurate expression in the stele and columella, ii) insertion of T-DNA in the plant genome is random during transformation which may affect gene expression in the obtained lines (Feng and Mundy, 2006) and as a consequence the intensity of expression conferred by the promoter (Schneeberger et al., 2005) resulting in impaired protein visualization in some tissues and iii) some variation in the techniques used in each laboratory.

We observed overlapping transcriptional expression in the organs and tissues for the four *A. thaliana sps* genes measured. These findings are in accord with studies that investigate the inactivation of these genes and show that when only one *sps*

gene is inactivated, it does not affect growth and reproduction of Arabidopsis plants. Only when there are double mutants (*AtSPS1F* and *AtSPS4F* genes) are the rosettes, flowers and siliques smaller than those of the wild-type plants. Moreover, the inactivation of one more gene (*AtSPS3F*) is required for plants to be sterile (Volkert et al., 2014; Bahaji et al., 2015). Consistently, the *AtSPS2F* gene showed the highest level of transcriptional expression in this work and does not affect growth and reproduction when it is inactivated (Volkert et al., 2014). Although the tissue transcription of the *sps* genes has diverged to theoretically fine tune their function, transcriptional overlapping may permit the plants to growth and reproduce without notable deficiencies when one *sps* gene is inactivated.

The expression of *sps* genes *AtSPS1F* and *AtSPS2F* in *A. thaliana* leaves is a common pattern in other species. In maize, only one of its seven *sps* genes is not expressed in this organ (Lutfylla et al., 2007). For rice, the major *sps* gene (*OsSPS1*) is expressed in the leaves (Chávez-Bárcenas et al., 2000). The above observations are associated with the availability of precursors for sucrose synthesis, mainly trioses derived of photosynthesis in the leaf cells (Ruan, 2004; Jiang et al., 2015). On the other hand, the expression was observed in the roots in three *sps* genes in *A. thaliana* and five *sps* genes in maize (Lutfylla et al., 2007), which suggests that sucrose synthesis is required in this organ. Moreover, although sucrose is transported to the root through the phloem, it has been suggested that in sink organs a cycle of breaking the disaccharide into glucose and fructose followed by re-synthesis should form sucrose to establish an appropriate balance in the distribution of carbohydrates entering cells for transport to other cells (Stitt and Sonnewald, 1995). The cycle of breaking and resynthesis of sucrose has been demonstrated in developing maize kernels (Cheng and Chourey, 1999). Therefore, we propose that, in *A. thaliana*, the synthesis of sucrose occurs in the stele region, where the *sps* expression was found, to permit the partitioning of carbohydrates to local cells and transport of sucrose to cortex cells.

The expression of the *AtSPS1F, AtSPS2F* and *AtSPS3F sps* genes in *A. thaliana* columella is interesting because it is the site of gravity perception by the roots. In these cells, it has been shown that amyloplasts function as statoliths when they sediment and activate potential receptors in the endoplasmic reticulum or plasmatic membrane, leading to a response of asymmetric growth in the root, involving the auxin hormone (Mendocilla et al., 2015). Starch accumulation in amyloplasts contributes to greater density and the ability to sediment. Thus, the synthesis of this polysaccharide is essential for these organelles to function as statoliths, a phenomenon demonstrated using mutants that synthesize less starch or lack this polysaccharide in the amyloplasts, which leads to a weaker response to gravity (MacCleery and Kiss, 1999). The expression of *A. thaliana sps* genes in columella suggests sucrose synthesis occurs in these tissue and sucrose synthesis induces the transcriptional expression of genes encoding ADP-glucose pyrophosphorylase (AGPase) the first committed enzyme in the starch synthesis in *A. thaliana* (Sokolov et al., 1998). In addition, it also induces *in vivo* starch synthesis (Tiessen et al., 2002). Hence, we hypothesize that the sucrose synthesis in the columella stimulates starch synthesis in this tissue.

Transcriptional expression in sepals and petals was shown for four *A. thaliana sps* genes, except for *AtSPS4F* whose expression additionally occurred in the developing embryos. Similarly, differences in the expression of the *sps* genes in reproductive structures have been found in other species. For example, *ZmSPS7* and *TaSPS2F* are expressed in embryos in maize and wheat, respectively (Lutfylla et al., 2007; Sharma et al., 2010). These results suggest that in each species, one of the *sps* genes has been selected for majority expression in embryos.

The osmotic stress established by our *in vitro* system allowed us to determine that the *A. thaliana sps* genes respond differentially to this factor. The transcriptional expression of *AtSPS2F* was increased more than four times and expression of *AtSPS4F* was duplicated. Additionally, *AtSPS1F* and *AtSPS3F* expression was not modified. Similarly, of the seven maize *sps* genes, the transcriptional expression of

ZmSPS3F was significantly induced in hydric stress conditions (Lutfylla et al., 2007). At the enzymatic level, considering the conserved amino acid sequence of spinach and maize, it has been predicted that in *A. thaliana*, the AtSPS1F, AtSPS2F and AtSPS4F enzymes are regulated by hydric stress, but not AtSPS3F (Lutfylla et al., 2007). Thus, there is coincidence in regulation by osmotic or hydric stress of the *AtSPS2F* and *AtSPS4F* genes and their corresponding encoded enzymes, but this is not the case for *AtSPS1F.* In maize, there is no complete correlation of gene transcriptional regulation by hydric stress, and the presence of potential regulatory sequences in the SPS enzymes (Lutfylla et al., 2007). Arabidopsis and maize data suggest that *sps* gene evolution of the promoter and coding regions does not necessarily determine its regulation by osmotic or hydric stress as transcriptional and enzymatic levels are comparably expressed.

The Increased sucrose synthesis under different abiotic stress conditions leads to osmotic stress. These findings suggest that this sugar and others participate as osmoprotectants in angiosperms (Drennan et al., 1993; Ingram et al., 1997; Strand et al., 1999) and in reducing reactive oxygen species (Keunen et al., 2013). In the *A. thaliana* line *abi4::uidA*, encoding a sensible genetic marker for the response to abscisic acid, reporter gene expression was not induced. Therefore, it is suggested that the transcriptional induction of *sps* Arabidopsis genes are not necessarily dependent on this hormone; instead, it is a primary response to osmotic stress.

Taken together, our results show the detailed expression of *A. thaliana sps* genes in organs and tissues. Our findings were consistent and complementary with previous reports by other groups. In addition, we demonstrated the differential transcriptional expression of *sps* genes in response to osmotic stress.

Figure Legends

Figure 1. Expression conferred by the promoters of the *sps* genes in *A. thaliana* vegetative structures. Homozygous seed lines transformed with each of the genetic constructs were germinated and grown for 10 days. The plants were processed as described in the Materials and Methods section to determine the colour derived from the *uidA* reporter gene expression. Photographs are representative of 15 plants analysed.

Figure 2. Expression conferred by the promoters of the *sps* genes from *A. thaliana* in stele and columella roots. Homozygous seed lines transformed with each of the genetic constructs were germinated and grown for 10 days. The plants were processed as described in the Materials and Methods section to determine the fluorescence emitted by GFP protein. Photographs are representative of 15 plants analysed.

Figure 3. Expression conferred by the promoters of the *sps* genes in *Arabidopsis thaliana* flowers. Homozygous seed lines transformed with each of the genetic constructs were germinated and grown in soil for 3-4 weeks. The collected flowers were processed as described in the Materials and Methods section to determine the colour derived from the *uidA* reporter gene expression. Photographs are representative of the 15 plants analysed.

Figure 4. Transcripts levels of the *Arabidopsis thaliana sps* genes. *A. thaliana* Col-0 seeds were germinated, and plants were grown in MS medium for 10 days. Total RNA was extracted, and the transcript levels were determined by qPCR as described in the Materials and Methods section. The levels of *sps* transcripts were standardized with the level of actin transcripts. The confidence interval for $\alpha = 0.05$ is indicated. Different letters indicate significant differences with the Tukey test ($P < 0.05$, $n = 3$).

Fig. 5. Effect of osmotic stress on the root growth and proline levels in *Arabidopsis thaliana.* Seeds of the *A. thaliana* Col-0 and the four SPS transgenic lines were germinated and grown for 10 days in different MS medias without (0 MPa) and with mannitol (-0.3 Mpa, -0.6 Mpa). The primary root growth was measured, and the amount of proline quantified as described in the Materials and Methods section. The confidence interval for $\alpha = 0.05$ is indicated. Different letters indicate significant differences with the Tukey test ($P < 0.05$, $n = 3$).

Figure 6. Effect of osmotic stress on the transcript levels of the *sps* genes in *Arabidopsis thaliana.* *A. thaliana* Col-0 seeds were germinated, and plants were grown for 15 days in different MS media without mannitol (0 MPa) and mannitol (-0.3 Mpa, -0.6 Mpa). Total RNA was extracted, and the transcript levels were determined by qPCR as described in the Materials and Methods section. The transcript levels of each *sps* gene of plants grown in mannitol were standardized with the respective *sps* transcript levels of plants grown in media without mannitol; additionally, all of the transcripts were standardized to the level of actin gene transcripts. The confidence interval for $\alpha = 0.05$ is indicated. Different letters indicate significant differences with the Tukey test ($P < 0.05$, $n = 3$).

Figure 7. Effect of osmotic stress on the expression of the *abi4::uidA* genetic marker in *Arabidopsis thaliana* plants. Seeds of the *A. thaliana abi4::uidA* line were germinated and grown for 72 h in MS media without mannitol (0 MPa) and mannitol (-0.3 Mpa, -0.6 Mpa). Plants were processed as described in the Materials and Methods section to determine the colour derived from the expression of the *uidA* reporter gene.

Figure 1S. Steps in the construction of vectors with the promoter regions of the *Arabidopsis thaliana sps* genes fused to the *gfp::uidA* reporter genes. A. Amplification of DNA fragments with the 5' regulatory regions of *A. thaliana sps* genes; lanes: 1 = *AtSPS1F*, 2 = *AtSPS2F*, 3 = *AtSPS3F*, 4 = *AtSPS4F*. B. *Escherichia coli DH5α* colonies transformed with the pDON221® plasmid with the

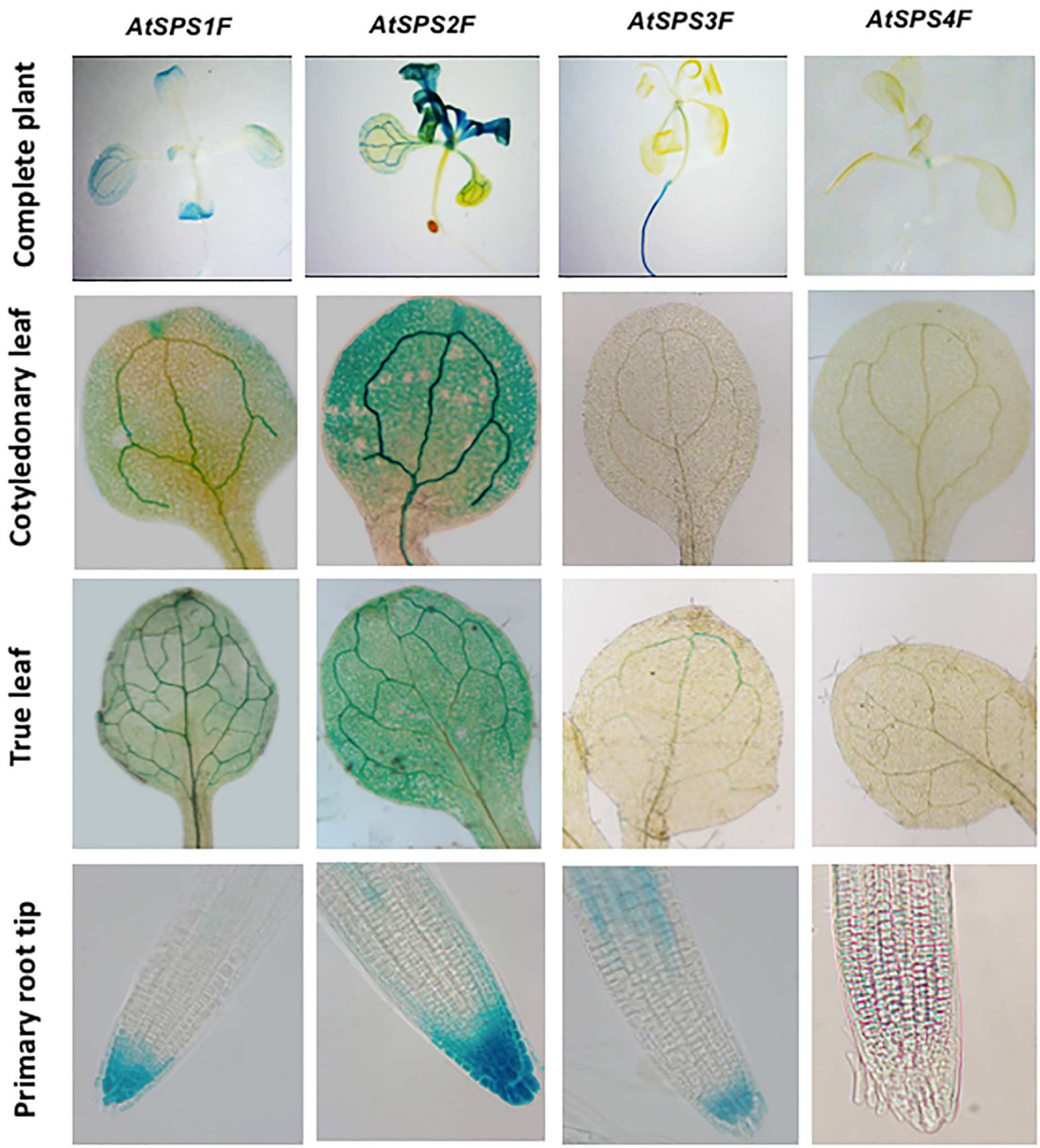
5´ *AtSPS1F* regulatory region, selected in kanamycin; transformation results with vectors carrying the other *sps* genes were similar. B. Colonies of *Agrobacterium tumefaciens* pGV2260 colonies transformed with pKGWF7 carrying the 5´ *AtSPS1F* regulatory region, selected in rifampin, streptomycin, spectinomycin and carbenicillin. The procedure for each step is described in the Materials and Methods section.

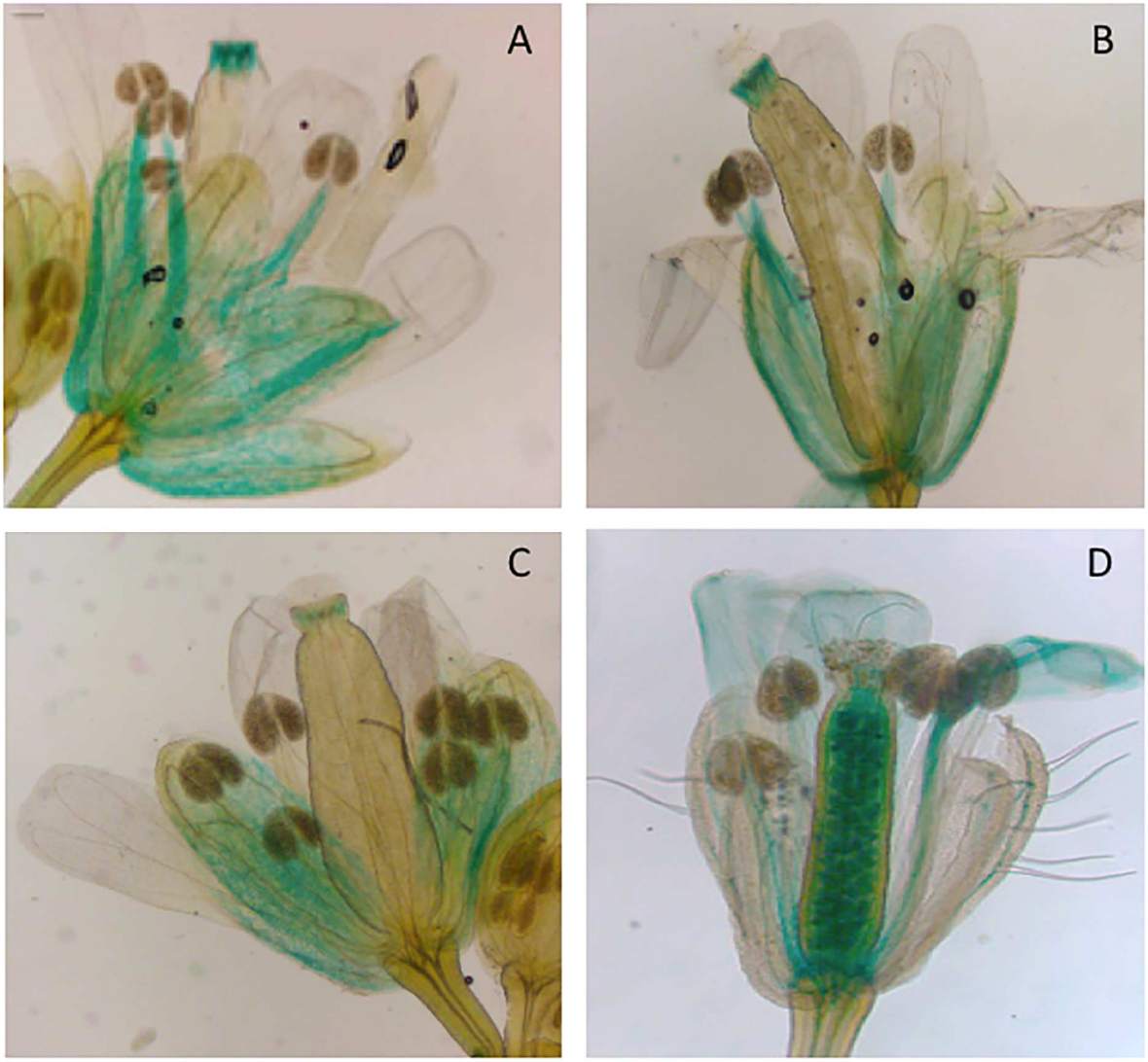
Figure 2S. Steps in the *Arabidopsis thaliana* transformation with the constructed *sps* vectors. A. Application of *Agrobacterium tumefaciens* suspension to closed flowers. B. Selection in kanamycin of T0 transformed plants. C. Selection in kanamycin of transformed T1 transformed plants. The procedure for each step is described in the Materials and Methods section.

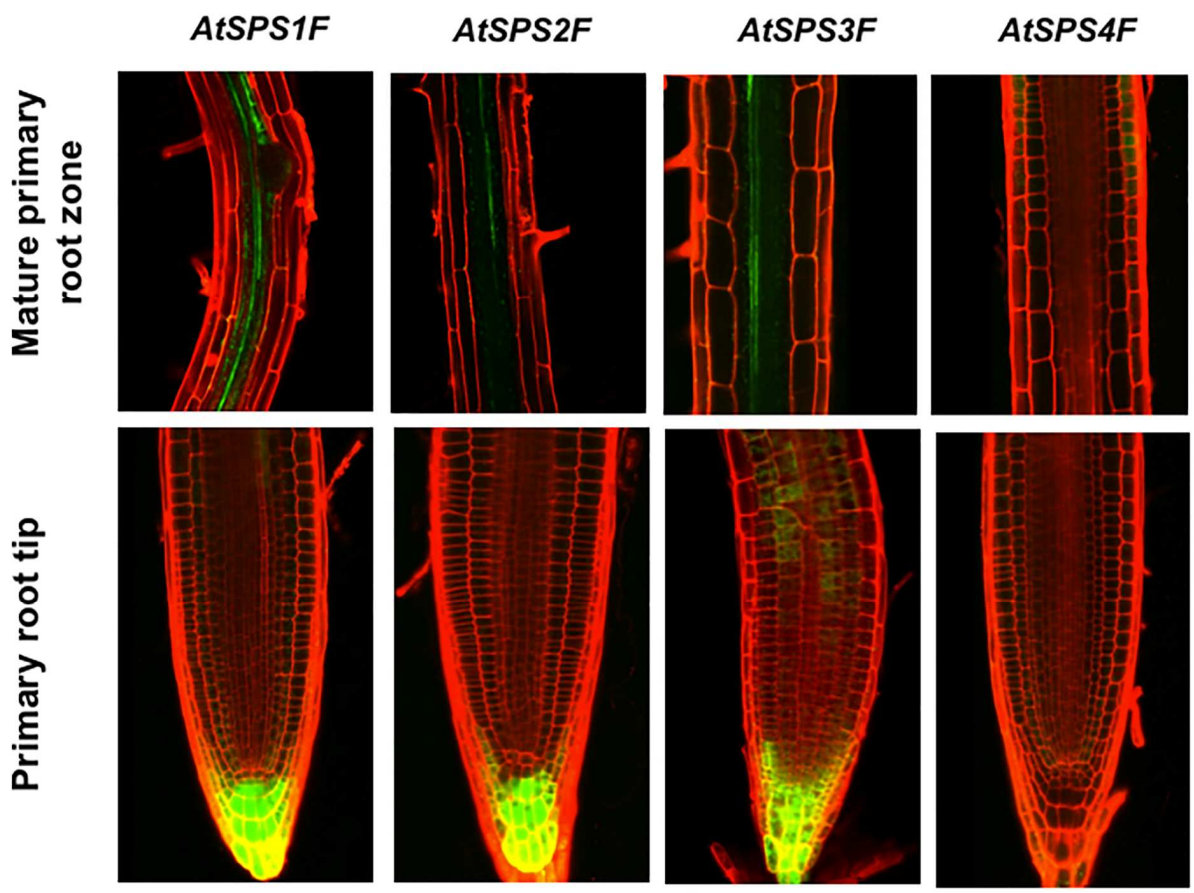
Table 1. Comparison of organ and tissue-specific expression patterns of the *sps* genes in *Arabidopsis thaliana* reported by Volkert et al. (2014) and in this work.

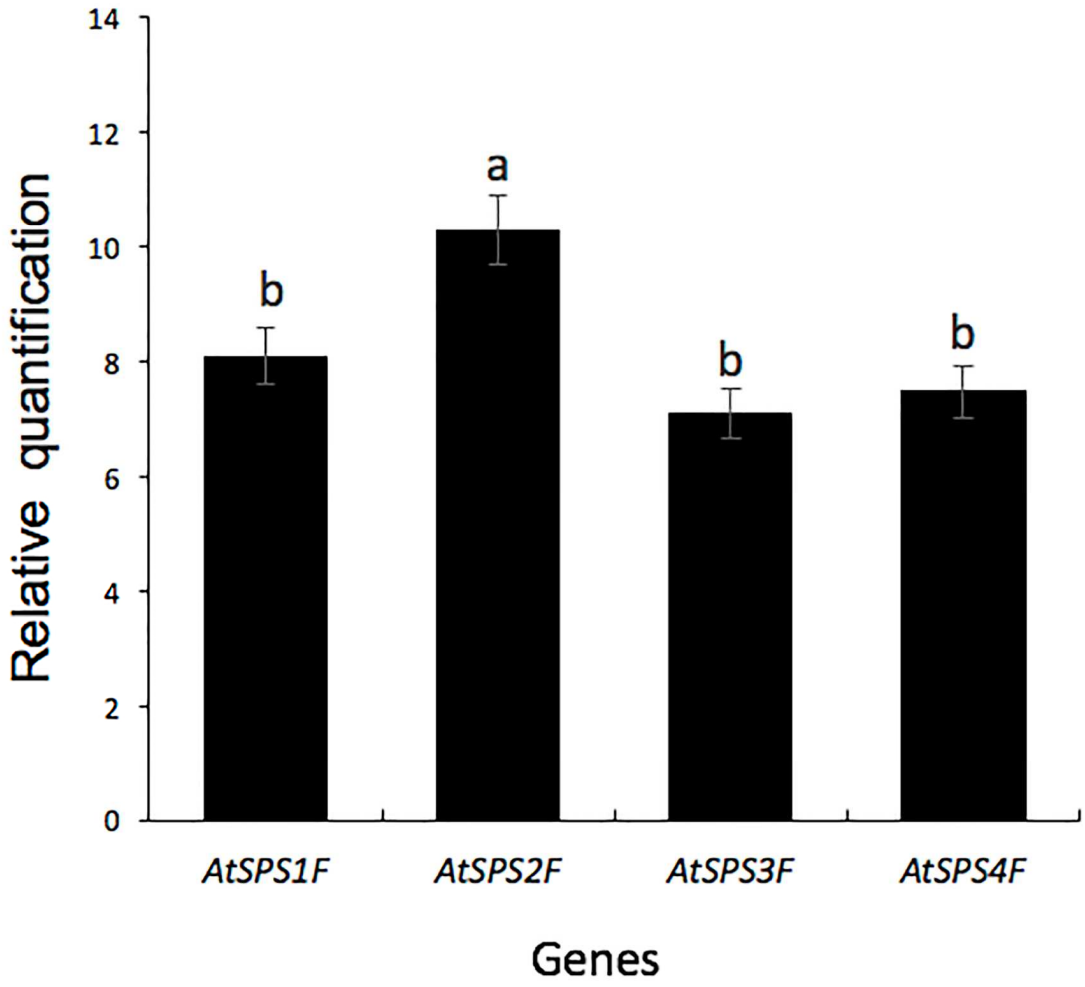
Genes	Expression reported	
	Volkert et al. (2014)	This work
AtSPS1F (*AtSPSA1*)	Cotiledonary leaves Mature leaves Stem Petals Anthers*	Cotiledonary leaves Mature leaves Stem Root stele** Columella** Sepals Petals
AtSPS2F (*AtSPSA2*)	Roots	Cotiledonary leaves** Mature leaves** Root stele Columella** Sepals** Petals**
AtSPS3F (*AtSPSB*)	Cotiledonary leaves* Stem* Anthers*	Root stele** Columella** Sepals** Petals**
AtSPS4F (*AtSPSC*)	Cotiledonary leaves* Mature leaves* Stem Petals Anthers*	Apical stem Sepals Petals Developing embryos**

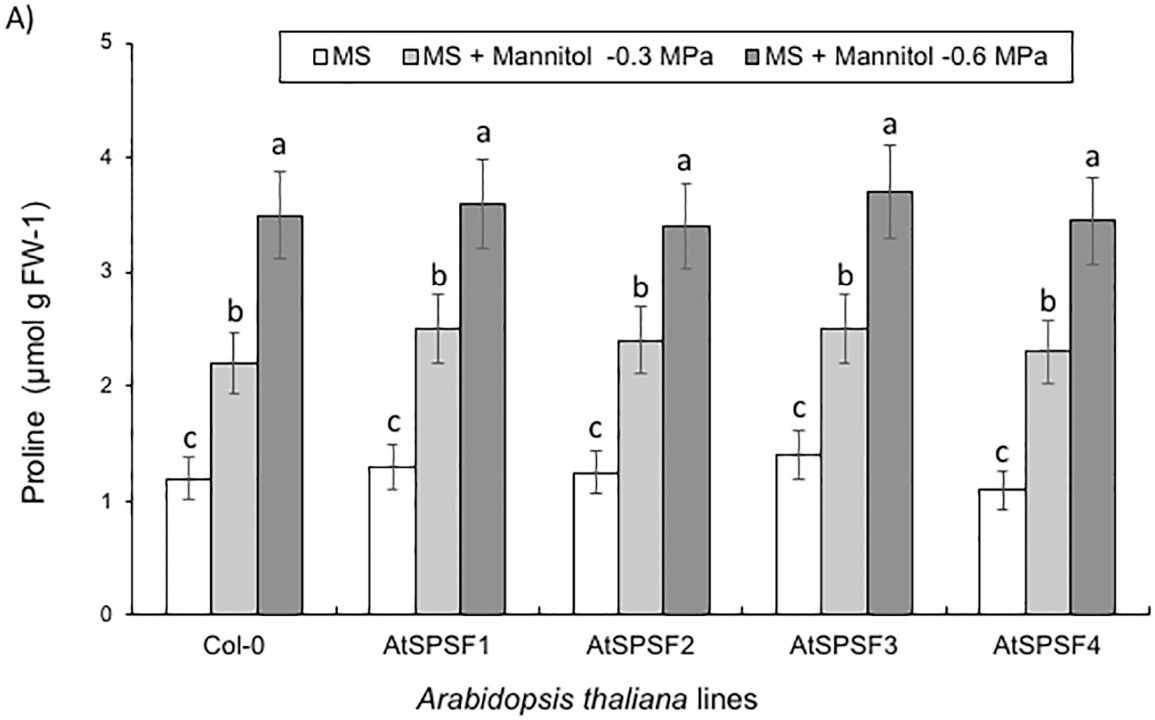
Expression reported by Volkert et al. (2014) not reported in this work (*).
Expression reported in this work, not reported by Volkert et al. (2014) (**).

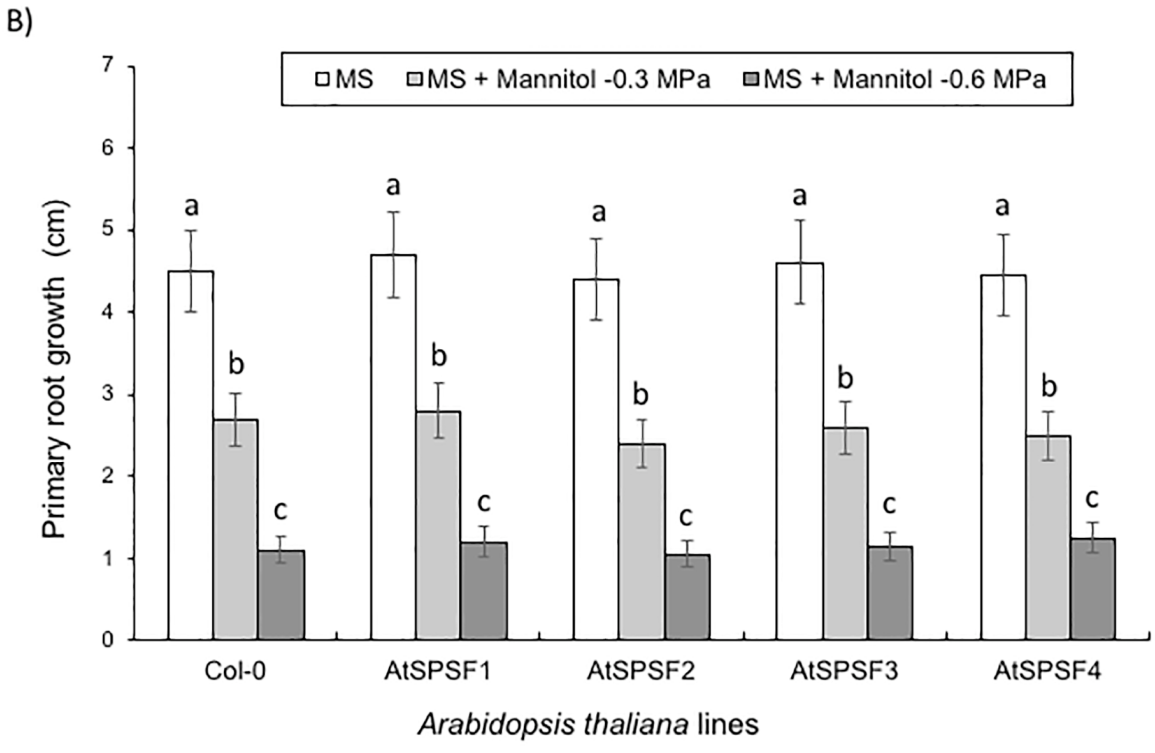

AtSPS1F
AtSPS2F
AtSPS3F
AtSPS4F
Complete plant
Cotyledonary leaf
True leaf
Primary root tip

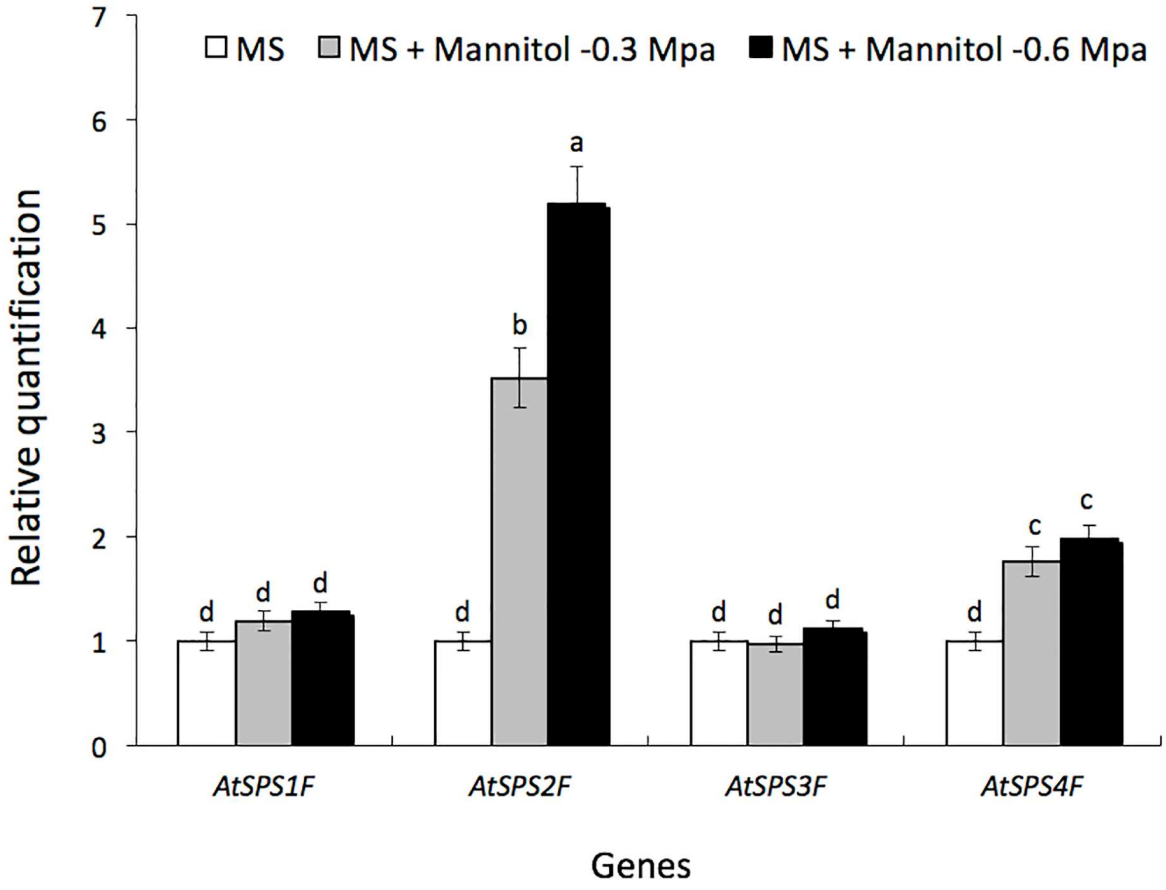


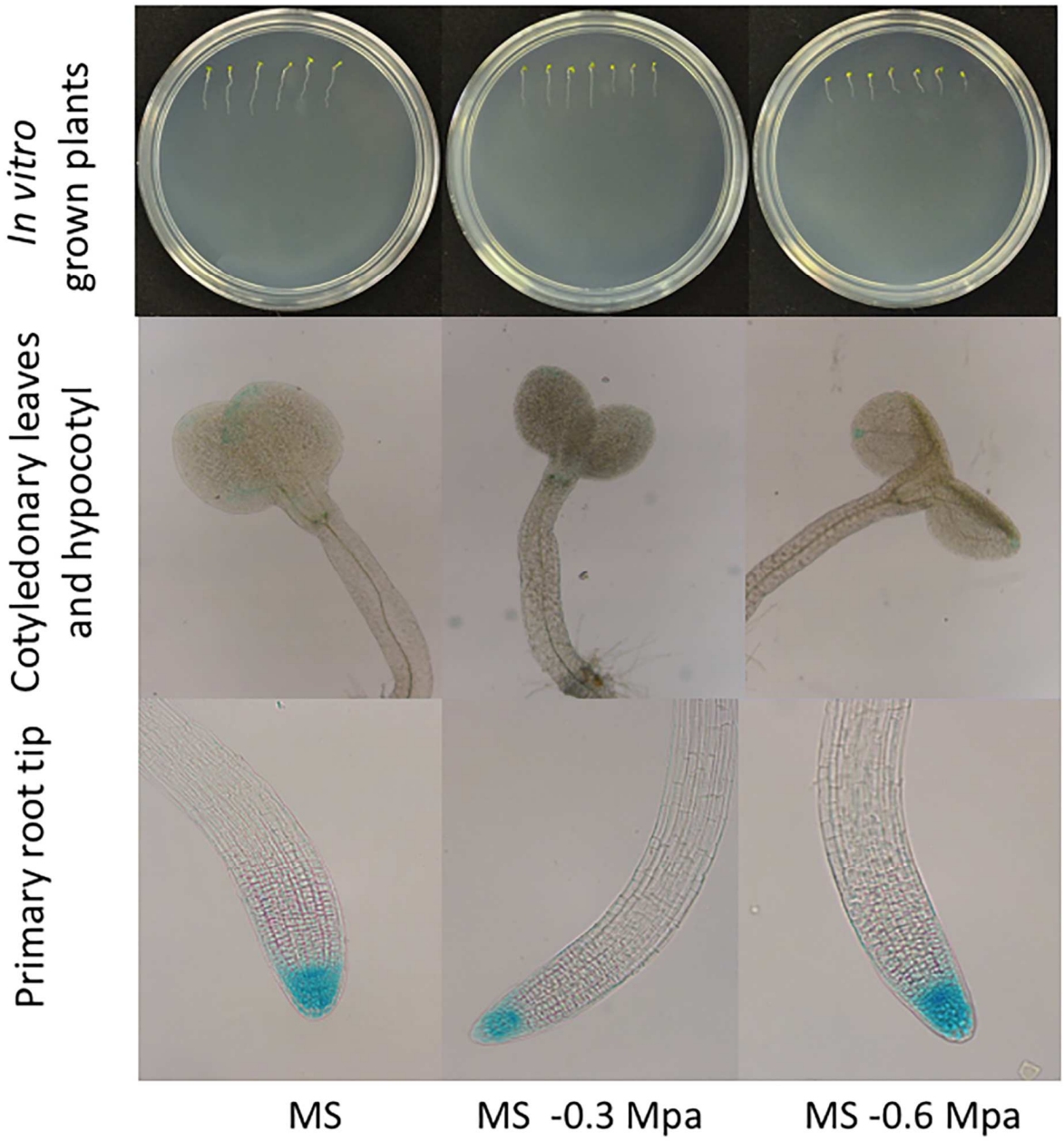


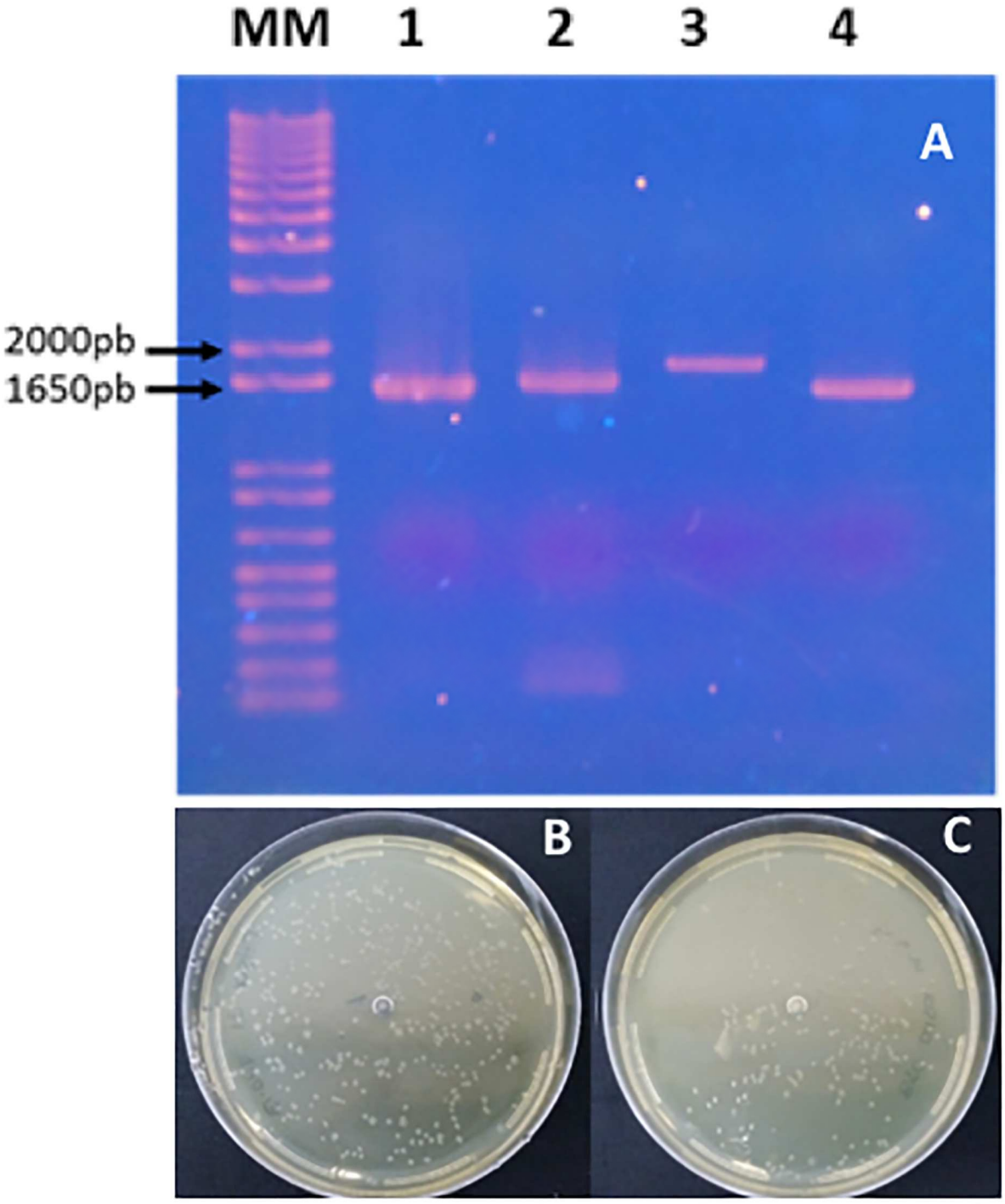

14
12
10
8
6
4
2
0
Relative quantification
a
b
b
b
AtSPS1F
AtSPS2F
AtSPS3F
AtSPS4F
Genes

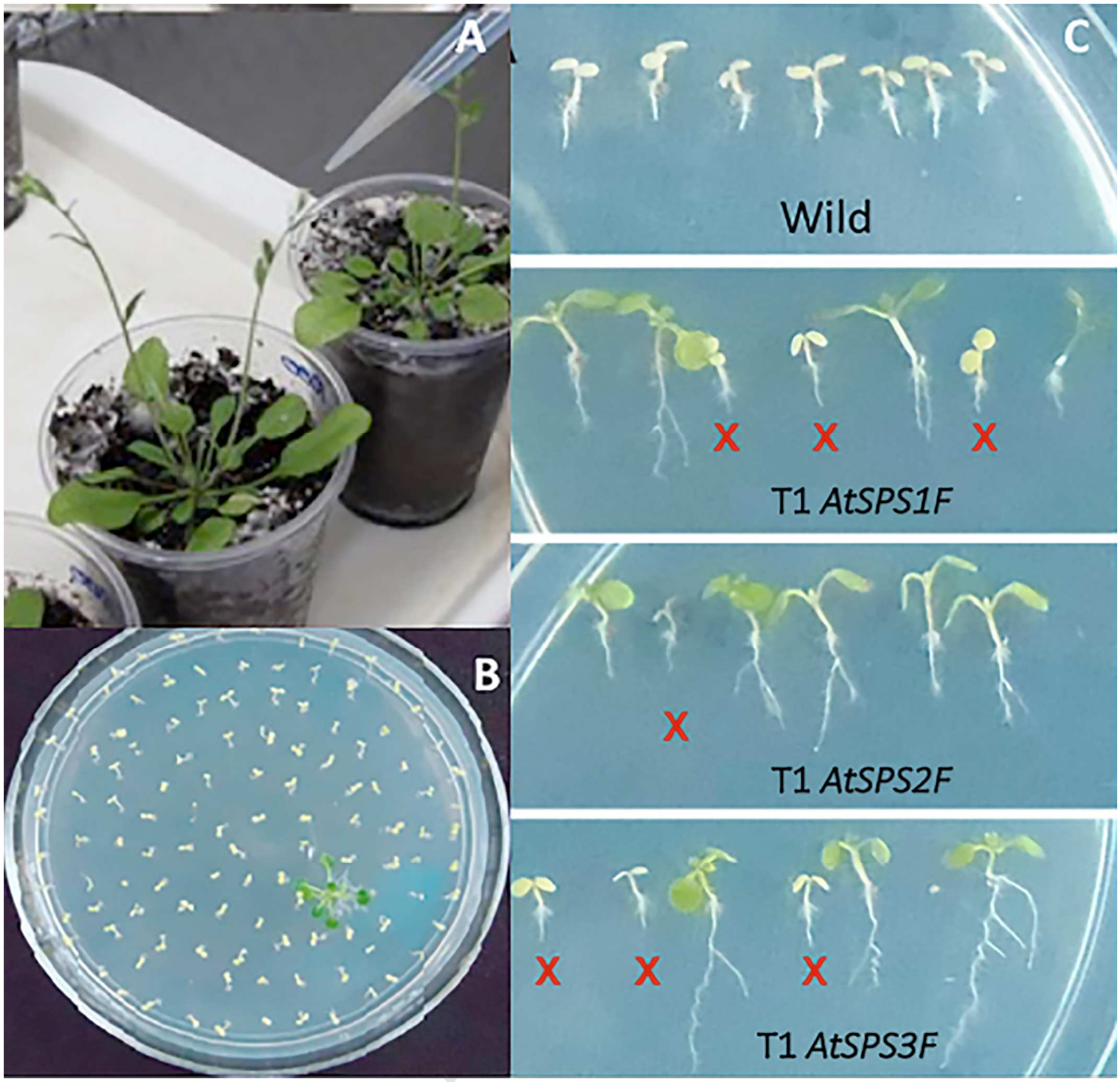

A)
MS
MS + Mannitol -0.3 MPa
MS + Mannitol -0.6 MPa
Proline (µmol g FW-1)
0
1
2
3
4
5
a
b
c
Col-0
AtSPSF1
AtSPSF2
AtSPSF3
AtSPSF4
Arabidopsis thaliana lines


B)
MS
MS + Mannitol -0.3 MPa
MS + Mannitol -0.6 MPa
Primary root growth (cm)
0
1
2
3
4
5
6
7
a
b
c
Col-0
AtSPSF1
AtSPSF2
AtSPSF3
AtSPSF4
Arabidopsis thaliana lines




In vitro grown plants
Cotyledonary leaves and hypocotyl
Primary root tip
MS
MS -0.3 Mpa
MS -0.6 Mpa


MM
1
2
3
4
A
2000pb
1650pb
B
C



REFERENCES

Amir, J., Preiss, J., 1982. Kinetic characterization of spinach leaf sucrose-phosphate synthase. Plant Physiol. 69, 1027-1030. http://dx.doi.org/10.1104/pp.69.5.1027.

Bahaji, A., Baroja-Fernández, E., Ricarte-Bermejo, A., Sánchez-López, A.M., Muñoz, F.J., Romero, J.M., Ruiz, M.T., Baslam, M., Almagro, G., Sesma, M.T., Pozueta-Romero, J., 2015. Characterization of multiple SPS knockout mutants reveal redundant functions of the four Arabidopsis sucrose phosphate synthase isoforms in plant viability, and strongly indicates that enhanced respiration and accelerated starch turnover can alleviate the blockage of sucrose biosynthesis. Plant Sci. 238, 135-147. http://dx.doi.org/10.1016/j.plantsci.2015.06.009.

Bates, L., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant and Soil. 39, 205-207. doi: 10.1007/BF00018060.

Bou-Torrent, J., Roig-Villanova, I., Martínez-García, J.F., 2008. Light signaling: back to space. Trends Plant Sci. 13, 108-114. http://dx.doi.org/10.1016/j.tplants.2007.12.003.

Castleden, C.K., Auk,i N., Gillespie, V.J., McRae, E.A., Quick, W.P,, Buchner, P., Foyer, C.H., Furbank, R.T., Lunn, J., 2004. Evolution and function of the sucrose-phosphate synthase gene families in wheat and other grasses. Plant Physiol. 135, 1753-1764. http://dx.doi.org/10.1104/pp.104.042457.

Chávez-Bárcenas, A.T., Valdez-Alarcón, J.J., Martinez-Trujillo, M., Chen, L., Xoconostle-Cázarez, B., Lucas, W.J., Herrera-Estrella, L., 2000. Tissue-specific and developmental pattern of expression of the rice *sps*1 Gene. Plant Physiol. 124, 641-653. http://dx.doi.org/10.1104/pp.124.2.641.

Cheng, W.H., Chourey, P.S., 1999. Genetic evidence that invertase-mediated release of hexoses is critical for appropriate carbon partitioning and normal seed development in maize. Theor Appl Genet. 98, 485-495. doi:10.1007/s001220051096.

Colón-Carmona, A., You, R., Haimovitch-Gal, T., Doerner, P., 1999. Spatio temporal analysis of mitotic activity with a labile cyclin–GUS fusion protein. Plant J. 20, 503-508. doi: 10.1046/j.1365-313x.1999.00620.x.

Do Nascimento, J.R., Cordenunsi, B.R., Lajolo, F.M., Alcocer, M.J., 1997. Banana sucrose-phosphate synthase gene expression during fruit ripening. Planta. 203, 283-288. https://dx.doi.org/10.1007/s004250050193.

Doehlert, D.C., Huber, S.C., 1983. Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate, and pH. Plant Physiol. 73, 989-994. http://dx.doi.org/10.1104/pp.73.4.989.

Drennan, P.M., Smith, M.T., Goldsworthy, D., Vanstaden, J., 1993. The occurrence of trehalose in the leaves of the desiccation-tolerant angiosperm *Myrothamnus flabellifolius* welw J. Plant Physiol. 142, 493-496. http://dx.doi.org/10.1016/S0176-1617(11)81257-5

Feng, C.P., Mundy, J., 2006. Gene discovery and functional analyses in the model plant *Arabidopsis*. J Integ Plant Biol. 48, 5-14. http://dx.doi.org/10.1111/j.1744-7909.2006.00205.x.

Fujita, Y., Fujita, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2011. ABA-mediated transcriptional regulation in response to osmotic stress in plants. J Plant Res. 124, 509-525. https://dx.doi.org/10.1007/s10265-011-0412-3.

Haseloff, J., Amos, B., 1995. GFP in plants. Trends Genet 11:328-329. doi:10.1016/0168-9525(95)90186-8.

Heim, U., Weber, H., Wobus, U., 1996. Cloning and characterization of full-length cDNA encoding sucrose phosphate synthase from faba bean. Gene. 178, 201-203. http://dx.doi.org/10.1016/0378-1119(96)00373-3.

Hesse, H., Sonnewald, U., Willmitzer, L., 1995. Cloning and expression analysis of sucrose-phosphate synthase from sugar beet (*Beta vulgaris* L.). Mol Gen Genet. 247, 515-520. doi:10.1007/BF00293155.

Huber, S.C., Huber, J.L., 1996. Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol. 47, 431-444. https://dx.doi.org/10.1146/annurev.arplant.47.1.431.

Ingram, J., Chandler, J.W., Gallgher, L., Salamini, F., Bartels, D., 1997. Analysis of cDNA clones encoding sucrose phosphate synthase in relation to sugar interconversions associated with dehydration in the resurrection plant *Craterostigma plantagineum* Hochst. Plant Physiol. 115, 113-121. http://dx.doi.org/10.1104/pp.115.1.113.

Jefferson, R.A., Kavanagh, T.A., Bevan, M., 1987. GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 6, 3910-3907. http://dx.doi.org/10.1016/0168-9525(95)90186-8.

Jiang, S.Y., Chi, Y.H., Wang, J.Z., Zhou, J.X., Cheng, Y.S., Zhang, B.L., Ma, A., Vanitha, J., Ramachandran, S., 2015. Sucrose metabolism gene families and their biological functions. Sci Rep. 5, 17583. https://dx.doi.org/10.1038%2Fsrep17583.

Kalt-Torres, W., Kerr, P.S., Huber, S.C., 1987. Isolation and characterization of multiple forms of maize leaf sucrose-phosphate synthase. Physiol Plant. 70, 653-658. doi: 10.1111/j.1399-3054.1987.tb04320.x.

Keunen, E., Peshev, D., Vangronsveld, J., Van Den Ende, W., Cuypers, A., 2013. Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant Cell Environ. 36, 1242-1255. http://dx.doi.org/10.1111/pce.12061.

Klein, R.R., Crafts-Brandner, S.J., Salvucci, M.E., 1993. Cloning and developmental expression of the sucrose-phosphate-synthase gene from spinach. Planta. 190, 498-510. doi:10.1007/BF00224789.

Komatsu, A., Takanokura, Y., Omura, M., Akihama, T., 1996. Cloning and molecular analysis of cDNAs encoding three sucrose phosphate synthases isoforms from a citrus fruit (*Citrus unshiu* Marc). Mol Gen Genet. 252, 346-351. doi: 10.1007/BF02173781.

Langenkämper, G., M.cHale, R., Gardner, R.C., MacRae, E., 1998. Sucrose-phosphate synthase steady-state mRNA increases in ripening kiwifruit. Plant Mol Biol. 36, 857-869. doi:10.1023/A:1005964812161.

Langenkämper, G., Fung, R.W., Newcomb, R.D., Atkinson, R.G., Gardner, R.C., MacRae, E.A., 2002. Sucrose phosphate synthase genes in plants belong to three different families. J Mol Evol. 54, 322-332. https://dx.doi.org/10.1007/s00239-001-0047-4.

Lutfylla, L.L., Xu, N., D'Ordine, R.L., Morrell, J.A., Miller, P.W., Duff, S.M., 2007. Phylogenetic and expression analysis of sucrose phosphate synthase isozymes in plants. J Plant Physiol. 164, 923-933. https://dx.doi.org/10.1016/j.jplph.2006.04.014.

MacCleery, S.A., Kiss, J.Z., 1999. Plastid sedimentation kinetics in roots of wild-type and starch-deficient mutants of *Arabidopsis*. Plant Physiol. 120, 183-192. http://dx.doi.org/10.1104/pp.120.1.183.

Malamy, J.E., Benfey, P.N., 1997. Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development. 124, 33-44. PMID: 9006065.

Martínez-Trujillo, M., Chávez-Bárcenas, T., Limones-Briones, V., Simpson, J., Herrera-Estrella, L., 2004. Functional analysis of the promoter of the rice sucrose phosphate synthase gene (*sps*1). Plant Sci. 166, 131-140. http://dx.doi.org/10.1016/j.plantsci.2003.08.017.

McBride, K.E., Summerfelt, K.R., 1990. Improved binary vectors for Agrobacterium mediated plant transformation. Plant Mol Biol. 14, 269-276. http://dx.doi.org/10.1007/BF00018567.

McMichael, R.W. Jr., Klein, R.R., Salvucci, M.E., Huber, S.C., 1993. Identification of the major regulatory phosphorylation site in sucrose-phosphate synthase. Arch Biochem Biophys. 307, 248-252. https://dx.doi.org/10.1006/abbi.1993.1586.

McMichael, R.W. Jr., Kochansky, J., Klein, R.R., Huber, S.C., 1995. Characterization of the substrate specificity of the sucrose-phosphate synthase protein kinase. Arch Biochem Biophys. 321, 71-75. http://dx.doi.org/10.1006/abbi.1995.1369.

Mendocilla, E., Hijazi, H., Bennet, M.J., Vissenverg, K., Swarum, R., 2015. New insights into root gravitropic signaling. J Exp Bot. 66, 2155-2165. http://dx.doi.org/10.1093/jxb/eru515.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15, 473-497. doi:10.1111/j.1399-3054.1962.tb08052.x.

Nielsen, T.H., Huber, S.C., 1989. Unusual regulatory properties of sucrose-phosphate synthase purified from soybean (*Glycine max*) leaves. Physiol Plant. 76, 309-314. doi:10.1111/j.1399-3054.1989.tb06196.x.

Okamura, M., Aoki, N., Hirose, T., Yonekura, M., Ohto, C., Ohsugi, R., 2011. Tissue specificity and diurnal change in gene expression of the sucrose phosphate synthase gene family in rice. Plant Sci. 181, 159-166. https://dx.doi.org/10.1016/j.plantsci.2011.04.019.

Oliveira, J.R., Cordenunsi, B.R., Lajolo, F.M., Caruso, M.J., 1997. Banana sucrose phosphate synthase gene expression during fruit ripening. Planta. 203, 283-288. https://dx.doi.org/10.1007/s004250050193.

Panchy, N., Lehti-Shiu, M., Shiu, S.H., 2016. Evolution of gene duplication in plants. Plant Physiol. 171, 2294-2316. http://dx.doi.org/10.1104/pp.16.00523.

Ruan, Y.L., 2014. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol. 65, 33-67. https://dx.doi.org/10.1146/annurev-arplant-050213-040251.

Salerno, G.L., Pontis, H.G., 1978. Sucrose phosphate synthetase. Separation from sucrose synthetase and a study of its properties. Planta. 142, 41-48. https://dx.doi.org/10.1007/BF00385118.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 74, 5463-5467. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC431765/.

Schneeberger, R.G., Zhang, K., Tatarinova, T., Troukhan, M., Kwok, S.F., Drais, J., Klinger, K., Orejudos, F., Macy, K., Bhakta, A., Burns, J., Subramanian, G., Donson, J., Flavell, R., Feldmann, K.A., 2005. Agrobacterium T-DNA integration in *Arabidopsis* is correlated with DNA sequence compositions that occur frequently in gene promoter regions. Funct Integr Genomics. 5, 240-253. https://dx.doi.org/10.1007/s10142-005-0138-1.

Sharma, S., Sreenivasulu, N., Harshavardhan, V.T., Seiler, C., Sharma, S., Khalil, Z.N., Akhunov, E., Sehgal, S.K., Röder, M.S., 2010. Delineating the structural, functional and evolutionary relationships of sucrose phosphate synthase gene family II in wheat and related grasses. BMC Plant Biol. 10, 134-147. https://dx.doi.org/10.1186/1471-2229-10-134.

Söderman, E., Brocard, I., Lynch, T., Finkelstein, R., 2000. Regulation and function of the *Arabidopsis* ABA-insensitive4 (ABI4) gene in seed and ABA response signaling networks. Plant Physiol. 124, 1752-1765. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC59872/.

Sokolov, L.N., Déjardin, A., Kleczkowski, L.A., 1998. Sugars and light/dark exposure trigger differential regulation of ADPglucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). Biochem J. 336, 681-687. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1219920/.

Sonnewald, U., Quick, W.P., MacRae, E., Krause, K-P., Stitt, M., 1993. Purification, cloning and expression of spinach leaf sucrose-phosphate synthase in *Escherichia coli*. Planta. 189, 174-181. doi:10.1007/BF00195074.

Stitt, M., Wilke, I., Feil, R., Heldt, W., 1988. Coarse control of sucrose phosphate synthase in leaves: Alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. Planta. 174, 217-230. https://dx.doi.org/10.1007/BF00394774.

Stitt, M., Sonnewald, U., 1995. Regulation of metabolism in transgenic plants. Annu Rev Plant Physiol Plant Mol Biol. 46, 341-368. https://dx.doi.org/10.1007/978-94-011-4818-4.

Strand, Å., Hurry, V., Henkes, S., Huner, N., Gustafsson, P., Gardestrom, P., Stitt, M., 1999. Acclimation of *Arabidopsis* leaves developing at low temperatures. Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin cycle and in the sucrose-biosynthesis pathway. Plant Physiol. 119, 1387-1397. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC32024/.

Sugiharto, B., Sakakibara, H., Sumadi., Sugiyama, T., 1997. Differential Expression of Two Genes for Sucrose-Phosphate Synthase in Sugarcane: Molecular Cloning of the cDNAs and Comparative Analysis of Gene Expression. Plant Cell Physiol. 38, 961-965. doi: 10.1093/oxfordjournals.pcp.a029258.

Tiessen, A., Hendriks, J.H., Stitt, M., Branscheid, A., Gibon, Y., Farré, E.M., Geigenberger, P., 2002. Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: A novel regulatory mechanism. Plant Cell. 14, 2191-2213. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC150765/.

Valdez-Alarcón, J.J., Ferrando, M., Salerno, G., Jimenez-Moraila, B., Herrera-Estrella, L., 1996. Characterization of a rice sucrose-phosphate synthase-encoding gene. Gene. 170, 217-222. http://dx.doi.org/10.1016/0378-1119(95)00854-3.

Volkert, K., Debast, S., Voll, L.M., Voll, H., Schießl, I., Hofmann, J., Schneider, S., Börnke, F., 2014. Loss of the two major leaf isoforms of sucrose-phosphate synthase in Arabidopsis thaliana limits sucrose synthesis and nocturnal starch degradation but does not alter carbon partitioning during photosynthesis. J Exp Bot. 65, 5217-5229. doi: 10.1093/jxb/eru282.

Winter, H., Huber, S.C., 2000. Regulation of sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes. Crit Rev Plant Sci. 19, 31-67. https://dx.doi.org/10.1080/10409230008984165.

Worrell, A.C., Bruneau, J.M., Summerfelt, K., Boersig, M., Voelker, T.A., 1991. Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. Plant Cell. 3, 1121-1130. http://dx.doi.org/10.1105/tpc.3.10.1121.

Xiong, J., Zhu, K., 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ. 25, 31-139. doi: 10.1046/j.1365-3040.2002.00782.x.

Yonekura, M., Aoki, N., Hirose, T., Onai, K., Ishiura, M., Okamura, M., Ohsugi, R., Ohto, C., 2013. The promoter activities of sucrose phosphate synthase genes in rice, OsSPS1 and OsSPS11, are controlled by light and circadian clock, but not by sucrose. Front Plant Sci. 4, 1-8. https://dx.doi.org/10.3389/fpls.2013.00031.