Plant Mol Biol Rep (2012) 30:123–130
DOI 10.1007/s11105-011-0323-4

Characterization of Three Sorbitol Transporter Genes in Micropropagated Apple Plants Grown under Drought Stress

Fang Li · Hengjiu Lei · Xiangjuan Zhao · Rongrong Tian · Tianhong Li

Published online: 1 June 2011

Abstract Sorbitol, a major end-product of photosynthesis in many species of the Rosaceae family, accumulates in response to abiotic stressors. However, the relationship that arises between the expression of sorbitol transporters and sorbitol accumulation under abiotic stress remains unclear. In this study, micropropagated 'Fuji' apple plants (*Malus domestica* Borkh. 'Fuji') were exposed to two varying degrees of osmotic stress and compared relative to an unstressed control. The osmotic stress was generated by adding PEG 6000 into full-strength Hoagland solution and adjusted the osmotic potential to either −0.75 MPa (mild drought stress [MIS]) or −1.5 MPa (severe drought stress [SES]). Analysis of sorbitol levels via high performance liquid chromatography (HPLC) showed that the sorbitol concentration was elevated in roots, phloem tissues and leaves in both the MIS and SES treatments compared to controls for the entire duration of the experiment. Three cDNA sequences, encoding sorbitol transporters (*MdSOT3*, *MdSOT4* and *MdSOT5*), were isolated from leaves. Real-time quantitative PCR (RT-qPCR) data suggests that the expression levels of *MdSOT3* and *MdSOT5* were higher under MIS and SES in roots, phloem tissues and leaves compared to unstressed controls. The average mRNA levels of *MdSOT4* in phloem tissues declined under both drought treatments (with the exception being at 2 h of SES). In roots and leaves under SES, mRNA production was increased. These results indicate that the up-regulation of *MdSOT3* and *MdSOT5* expression is consistent with the accumulation of sorbitol under conditions of osmotic stress in apple plants. They enhanced drought tolerance in vegetative tissues. Increased *MdSOT4* mRNA enhanced drought tolerance under SES.

Keywords Drought stress · Relative water content · Sorbitol transporter · Sorbitol content

F. Li · H. Lei · X. Zhao · R. Tian · T. Li (✉)
Department of Fruit Science, College of Agronomy and Biotechnology, China Agricultural University,
2 Yuanmingyuan West Road, Haidian District,
Beijing 100193, China
e-mail: lith@cau.edu.cn

F. Li · H. Lei · X. Zhao · R. Tian · T. Li
Key Laboratory of Stress Physiology and Molecular Biology for Fruit Trees of Beijing, China Agricultural University,
2 Yuanmingyuan West Road, Haidian District,
Beijing 100193, China

Introduction

Drought stress is one of the main abiotic stressors that limit plant growth and production (Yang et al. 2010; Wang et al. 2011; Maqbool et al. 2009). Water deficiency is a threat to agriculture because of the inability to control water availability without the use of irrigation strategies (Prabu et al. 2011). Polyols, with their low molecular weight, high solubility and non-reducibility, are suitable substrates for translocation enzymes and their accumulation results in improved tolerance to abiotic stress (Noiraud et al. 2001).

Sorbitol is the main polyol that is produced by the Rosaceae family, whose members include almond (*Prunus dulcis*), apple (*M. domestica*), cherry (*P. cerasus*), peach (*P. persica*) and pear (*Pyrus communis*) (Loescher and Everard 1996; Soria-Guerra et al. 2011). Numerous studies have shown that abiotic stress induces the accumulation of sorbitol. In mature apricot (*P. armeniaca*) leaves, up to 65–75% of translocated carbon is from sorbitol. In the phloem of apples, sorbitol comprises about 80% of translocated carbohydrates (Kühn et al. 1999; Lalonde et al. 2003).

Osmotic stress promotes sorbitol accumulation in the leaves and roots of loquat (*Eriobotrya japonica*) trees, and sorbitol content diminishes gradually once the osmotic stress is alleviated (Cui et al. 2003). Low temperature and drought stress also led to an increased concentration of sorbitol in peach leaves (Deguchi et al. 2002; Cui et al. 2004).

Many studies focused on the regulation of sugar transporter levels in plants subjected to abiotic stress. It has been shown that different sugar transporters are up-regulated or down-regulated by wounding, elicitor treatment, pathogen infection (Truernit et al. 1996), salt stress (Pommerrenig et al. 2007; Alexandra et al. 2006), drought stress (Alexandra et al. 2006), light (Köhn et al. 1997; Stadler et al. 2003) and phytohormones (Ehneβ and Roitsch 1997; Fillion et al. 1999). mRNA levels of *STP4,* which encodes an enzyme that catalyzes monosaccharide import into classic sinks, increased when Arabidopsis was wounded. The function of increased *STP4* concentration is to meet the increased carbohydrate demand of cells responding to environmental stress. When celery plants were subjected to salt stress conditions, they favored the accumulation of mannitol (Truernit et al. 1996). In *Plantago major*, the mRNA levels of vascular sorbitol transporters *PmPLT1* and *PmPLT2* were up-regulated in phloem sap following salt treatment (Pommerrenig et al. 2007). The studies cited above indicate that different sugar transporters each have their own unique response to environmental stress. For this reason, many studies are focusing on polyol transporters in response to different stressors. Little is known about the relationship of sorbitol transporters and sorbitol accumulation in members of the Rosaceae family subjected to drought stress. Sorbitol transporters have been identified in various plants including sour berry (*P. cerasus*), apple (*M. domestica*), common plantain (*P. major*) and Arabidopsis (*Arabidopsis thaliana*) (Gao et al. 2003, 2005; Watari et al. 2004; Fan et al. 2009). *MdSOT1* and *MdSOT2* were cloned from apples while *MdSOT3, MdSOT4*, *MdSOT5* and *MdSOT6* were isolated from apple source leaves. Expression of *MdSOT1* and *MdSOT2* was seen in all sink tissues except watercore-affected fruit tissues. The K_m values of MdSOT1 and MdSOT2 were 1.0 and 7.8 mM, respectively (Gao et al. 2005). Apparent K_m values of MdSOT3 and MdSOT5 for sorbitol were estimated to be 0.71 and 3.2 mM, respectively. *MdSOT3*, *MdSOT4* and *MdSOT5* were expressed mostly in vegetative organs while fruits showed little or only weak expression of *MdSOTs*. Interestingly, *MdSOTs* expression increased with leaf maturation (Watari et al. 2004).

To understand if the expression levels of sorbitol transporter genes contribute to sorbitol accumulation and drought tolerance, we investigated the relationship between *MdSOT3*, *MdSOT4* and *MdSOT5* transport levels and the sorbitol content in roots, phloem and leaves. These tissues were isolated from micropropagated apple plants and subjected to different drought intensities by regulating the osmotic potential of PEG 6000 solutions.

Materials and Methods

Plant Material, Growth and Experimental Conditions

Micropropagated 'Fuji' apple plants (*Malus domestica* Borkh. 'Fuji') were pre-cultured in pots with 1/2 Hoagland nutrient solution for 10 days and transferred into Hoagland solution. The culture conditions were 24–26°C, 60% relative humidity under 10 h/14 h (day/night). Plants with heights ranging from 25 to 30 cm and with uniform growth were selected for the study.

Plant Material Treatments

PEG 6000 was used as the drought stress factor as reported by Hsiao (1973). The control plants received Hoagland solution with sterile water in place of PEG 6000 (CK). Nutrient solutions contributing mild stress (mild drought stress [MIS]) and severe stress (severe drought stress [SES]) were adjusted to osmotic potentials of −0.75 and −1.5 MPa, respectively. A randomized complete block design was used, with three replicates consisting of 108 plants per block. Samples were collected at 0, 2, 6, 12, 24 and 48 h following the experimental drought stress treatments and control treatment (CK). Plant materials were separated into leaves and phloem preps of stems and roots. Leaf numbers 9, 10 and 11 were collected from the shoot apex (leaf number 1 was the youngest and number 20 was the oldest). All leaves had an approximate mass of 0.1 g. The phloem preps of the stem were collected from leaf numbers 6 to 14 excluding the bark. Additionally, young roots were cut and collected. The samples, which were used for RT-qPCR, were immediately transferred into liquid nitrogen and stored at −80°C. Samples for HPLC and relative water content (RWCs were used as soon as they were collected.

Analysis of Relative Water Content

The RWC of the micropropagated apple leaves was measured as previously described by Yamasaki and Dillenburg (1999). Source leaves were collected and immediately weighed (fresh mass [FM]). The leaves were then submerged in distilled water inside a closed petri dish at room temperature until solutions became saturated (turgid mass, TM). Leaf samples were placed in a heated in oven at 80°C for 48 h in order to obtain the dry mass (DM). Values of FM, TM, and DM were used to calculate RWC using the following equation: $\mathrm{RWC}(\%) = [(\mathrm{FM} - \mathrm{DM})/(\mathrm{TM} - \mathrm{DM})] \times 100$.

Apple RNA Extraction and Cloning of Sorbitol Transporter Genes

For RT-qPCR, the RNA of samples from leaves, phloem and roots were extracted using the hot borate protocol, according to Wan and Wilkins (1994). cDNA sequences of the three sorbitol transporters were amplified from total RNA isolated from micropropagated apple leaves with gene-specific primers. The primers were designed with Primer 5.0 according to the reported sequences in NCBI. The primer sequences are as follows: SOT3f: 5′-AGAGCATGACGGCAGTGGAC-3′ SOT3r: 5′-ATCCTCTGGAGATTCACACACAA-3′, SOT4f: 5′-ATCGGCACCACTAACTTATCTCC-3′, SOT4r: 5′-TAACCTGTTCCGCTGTCTGC-3′, SOT5f: 5′-AAGATGGCTGACCGGACAACT-3′, SOT5r: 5′-AGCAGAGTAAGACGAGGAACATA-3′. PCR was performed at 94°C for 5 min, and then incubated using a multi-step program (94°C, 30 s; 60°C, 40 s; 72°C, 50 s) for 30 cycles.

Analysis of Sorbitol Content in Micropropagated Apple Plants

Sorbitol was extracted from micropropagated apple leaves, phloem and roots according to the protocol published by Li and Li (2007a), and sorbitol content was determined by HPLC. Separations were done by chromatography with an SCR-101 C column (7.9×30 mm ID, 5 μm) (Shimadzu Corporation, Kyoto, Japan). The column was placed in an oven set to 45°C. The injection volume was 20 μl for all the runs. The HPLC system consisted of a WATERS-410 series pumping system (Shimadzu Corporation) fitted with a WATERS-2996 PDA detector. The flow rate of the solvent was adjusted to 1 ml/min and consisted of degassed, distilled water.

Analysis of *MdSOT3, MdSOT4* and *MdSOT5* by Real-Time Quantitative PCR

The probing primers for RT-qPCR were designed with Primer 5.0 software. The internal standard used was the apple 18S ribosomal RNA gene. The primer sequences used are as follows: MdSOT3f: 5′-CGTTGTTACTGACCAGCGTG-3′, MdSOT3r: 5′-CCAGGGGACTGAACCTTTGT-3′, MdSOT4f: 5′-GTGGAGAAAAGCCAATAAACTG-3′, MdSOT 4r: 5′-ATATTCGCTCACTAAGCCAAGA-3′, MdSOT5f: 5′-GATAATACAGTCTCCAGCCAACC-3′, MdSOT5r: 5′-CCAAAATAGCACAAGCAAAGG-3′, 18 s f: 5′-AAACGGCTACCACATCCA-3′, 18 s r: 5′-CACCAGACTTGCCCTCCA-3′. The total volume of each RT-qPCR reaction was 50 μl: 25 μl of 2× Hotstart Fluo-PCR mix, 1.0 μl of 3′ primers and 5′ primers (25 μM), 2 μl of cDNA and 20.7 μl of DEPC-treated ddH_2O. All amplicons had varying lengths within 250–300 bp. PCR was performed with a FTC-2000 rapid thermal cycler (Funglyn, Toronto, Canada) and all reactions were prepared with the Shine Probe RT-qPCR Master Mix Kits (Shine, Shanghai). PCR reaction conditions are as follows: 94°C for 4 min and 40 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, 72°C for 10 min.

Statistical Analysis

All analyses were performed with version 13.0 of the SPSS software. Differences between treatments were analyzed by Duncan's multiple range test at a probability level of 0.05.

Results

Changes in the RWC of Micropropagated Apple Leaves under Drought Stress

RWC remained at stable, high levels, between 89% and 92%, in CK leaves during the treatment period (Fig. 1) while the RWC of drought stress plants declined rapidly within 12 h (from 90% to 65% in MIS and from 90% to 48% in SES, respectively). Between 12 h and 48 h, the RWC declined only slightly. The RWC of apple leaves under MIS and SES remained at 60% and 42% at the end of the treatment, respectively.

Alterations in Sorbitol Content in Response to Drought Stress

The sorbitol content of micropropagated apple roots, phloem and leaves under the two experimental drought stress treatments increased rapidly (Fig. 2). In roots, this increase was significant within 2 h of the MIS and SES

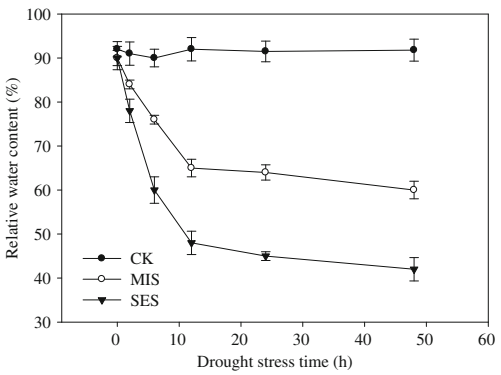

Fig. 1 Effects of drought stress on RWC of micropropagated apple plant leaves. Samples were collected at 0, 2, 6, 12, 24 and 48 h. Values are mean ± SE for three replicates. Statistically significant differences among treatments at each time are indicated by *vertical bars* ($P<0.05$). *Filled circle* normal water conditions (CK), *empty circle* mild drought stress (MIS), *inverted filled triangle* severe drought stress (SES)

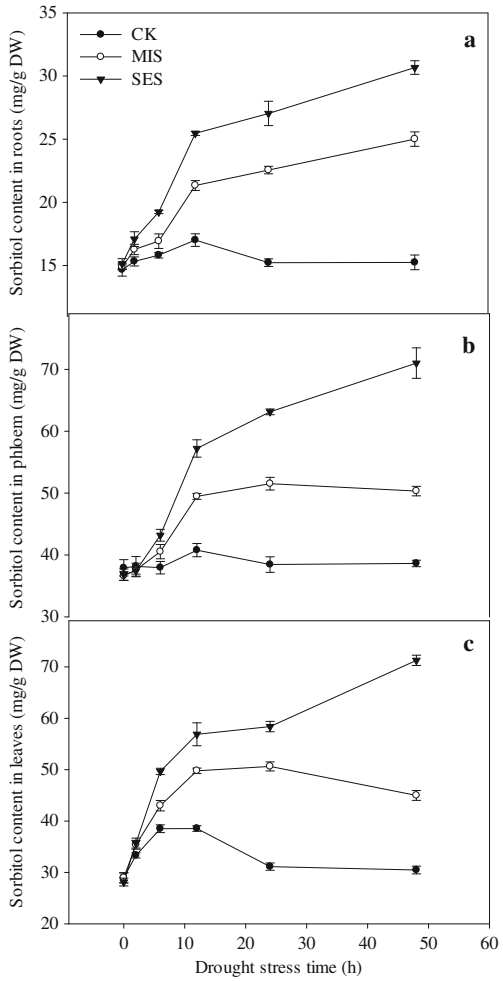

Fig. 2 Sorbitol content in roots, phloem and leaves of micropropagated 'Fuji' apple plants subjected to varying degrees of drought stress. Values are mean ± SE for three replicates. Statistically significant differences among treatments at each time are indicated by *vertical bars* ($P<0.05$). *Filled circle* normal water conditions (CK), *empty circle* mild drought stress (MIS), *inverted filled triangle* severe drought stress (SES)

treatments and maintained a steady increased rate up to 12 h, particularly under SES. At the end of drought stress treatment, the sorbitol content of the MIS and SES groups in roots was 25 and 30.7 mg/g DW, respectively, while the content was 15.2 mg/g DW in CK roots (Fig. 2a). In phloem, significant differences compared to CK were observed at 6 h following drought stress treatments (Fig. 2b). Sorbitol content in phloem under MIS (50 mg/g DW) and SES (71 mg/g DW) were much higher than controls (38.6 mg/g DW) at the end of the treatments. Significantly higher contents of sorbitol were found in SES leaves compared to MIS leaves at 6 h after initiating the drought stress, and these differences were maintained throughout the course of the experiment. The sorbitol content of leaves under MIS and SES was much higher than that with CK treatment at 48 h (1.3- and 1.8-fold compared to CK levels, respectively) (Fig. 2c).

Changes in Sorbitol Transporter Gene Expression under Drought Stress

The sequences of the sorbitol transporter genes isolated from apple leaves were consistent with the reported gene sequences in NCBI: *MdSOT3* (AB125646.1), *MdSOT4* (AB125647.1), and *MdSOT5* (AB125648.1). RT-qPCR results indicate that the expression levels of *MdSOT3* during drought stress treatments increased rapidly and were significantly higher compared to CK in all organs (Fig. 3). This increased was more pronounced in plants treated with SES. The expression levels of *MdSOT3* in roots remained at

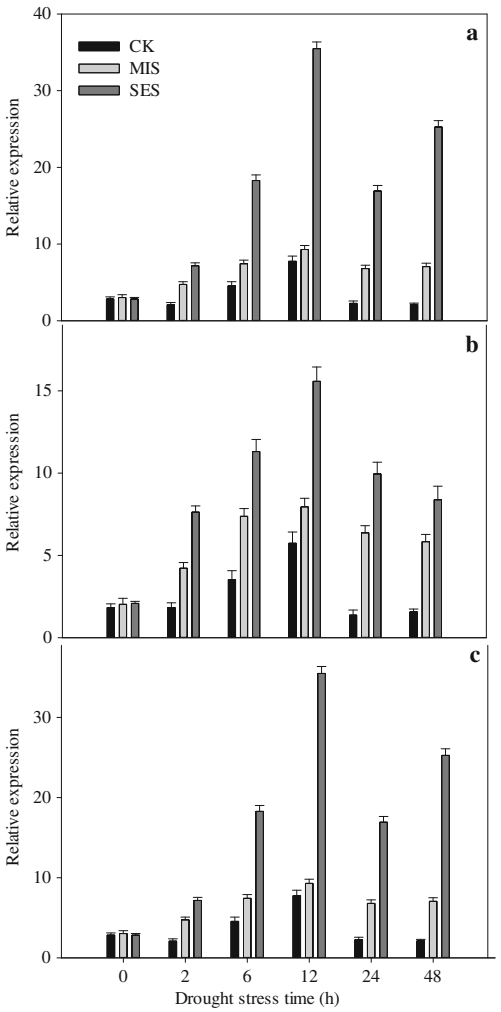

Fig. 3 Effect of drought stress on *MdSOT3* gene expression in micropropagated 'Fuji' apple plant roots (**a**), phloem (**b**) and leaves (**c**). Values are mean ± SE for three replicates. Statistically significant differences among treatments at each time are indicated by *vertical bars* ($P<0.05$). *Filled circle* normal water conditions (CK), *open circle* mild drought stress (MIS), *inverted filled triangle* severe drought stress (SES)

high levels after 6 h under SES and MIS, but in the CK treatment, these levels dropped to initial values after 24 h. The mRNA of *MdSOT3* in roots achieved the highest level under SES at 12 h, and this was 4.6-fold higher than that in CK treatment. At the end of the drought stress treatments, *MdSOT3* expression levels in MIS and SES were 2.3- and 11.9-fold compared to controls, respectively (Fig. 3a). The changes in *MdSOT3* expression levels in apple phloem and leaves were similar to those in roots (Fig. 3b and c).

MdSOT4 expression in roots was paralleled by that of *MdSOT3* (Fig. 4a), but the mRNA levels of *MdSOT4* were much higher than those of *MdSOT3* and *MdSOT5*. *MdSOT4* expression, however, declined in phloem under MIS throughout the course of the experiment. At 6 and 12 h, *MdSOT4* in phloem was reduced to 91% and 71% of CK treatment levels under SES (Fig. 4b). The most striking alteration was noted in phloem: the expression was dramatically increased at 24 and 48 h (about 1.6-fold compared to the CK treatment). No obvious change between *MdSOT4* expression levels of phloem under SES at 24 and 48 h was observed (Fig. 4b).

The expression levels of *MdSOT4* in leaves were reduced under MIS at 6 h and 12 h but was increased at all other time points. As opposed to the *MdSOT4* expression levels in phloem, the transcription levels of *MdSOT4* in leaves had no significant changes prior to 12 h but increased at 24 and 48 h under MIS (Fig. 4c). *MdSOT4* mRNA levels started to increase 2 h post-SES treatment until the end of the experimental run (Fig. 4c). The expression levels of *MdSOT5* (Fig. 5) under MIS paralleled the changes in *MdSOT3* expression. *MdSOT5* expression

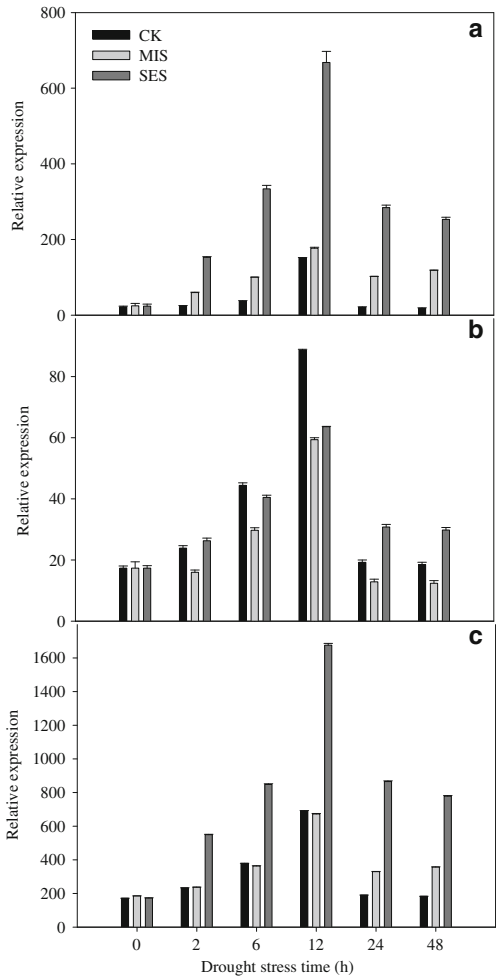

Fig. 4 Effect of drought stress on *MdSOT4* gene expression in micropropagated 'Fuji' apple plant roots (**a**), phloem (**b**) and leaves (**c**). Values are mean ± SE for three replicates. Statistically significant differences among treatments at each time are indicated by *vertical bars* ($P<0.05$). *Filled circle* normal water conditions (CK), *open circle* mild drought stress (MIS), *inverted filled triangle* severe drought stress (SES)

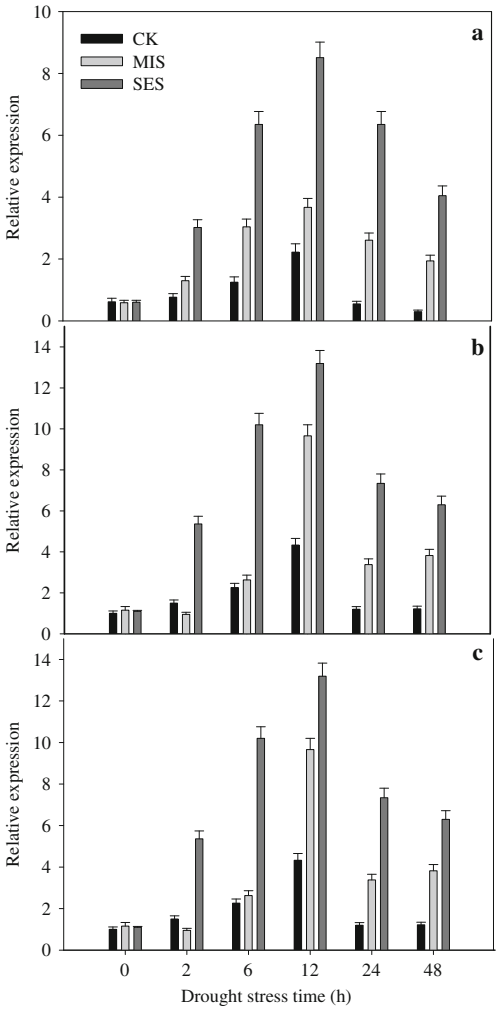

Fig. 5 Effect of drought stress on *MdSOT5* gene expression in micropropagated 'Fuji' apple plant roots (**a**), phloem (**b**) and leaves (**c**). Values are mean ± SE for three replicates. Statistically significant differences among treatments at each time are indicated by *vertical bars* ($P<0.05$). *Filled circle* normal water conditions (CK), *empty circle* mild drought stress (MIS), *inverted filled triangle* severe drought stress (SES)

levels decreased under MIS after 2 h in phloem and leaves, then gradually increased with persistent drought stress (Fig. 5b). The expression pattern of *MdSOT5* in SES (Fig. 5) was similar to that of *MdSOT3*.

The results indicate that the expression level of sorbtiol transporter genes, *MdSOT3, MdSOT4* and *MdSOT5*, is enhanced under conditions of SES with the exception of *MdSOT4* transcript levels in phloem at 6 h and 12 h (Fig. 4b). Additionally, the expression levels of *MdSOT3* and *MdSOT5* were induced by MIS at 6 h until 48 h. Only the expression level of *MdSOT4* in phloem decreased under MIS at all times compared to the CK treatment. Initially, under SES, *MdSOT4* expression was slightly elevated (1.1-fold compared to the CK treatment). The expression decreased from 6 to 24 h and remained elevated after 24 h when the SES treatment persisted (Fig. 4b).

Discussion

Annually, yield losses due to drought stress exceed the sum of other abiotic stresses (Su et al. 2010). Drought is one of the main environmental factors limiting crop production and plant distribution worldwide (Stolf-Moreira et al. 2011). Polyols are efficient osmolytes, and their accumulation is related to resistance to abiotic stressors (drought, salt and cold) (Hu et al. 2005; Rejskova et al. 2007) and biotic stresses (Stoop et al. 1996). Drought stress potentially disrupts the sorbitol distribution in different organs as a defense mechanism to a disadvantageous environment (Li and Li 2007a). Many plant physiological processes are influenced by abiotic stressors (Gao et al. 2010; Xu et al. 2001; Zhang et al. 2011).

Compared to CK treatment, the sorbitol content of micropropagated apple plants increased under MIS and SES to different extent (Fig. 2), and these observations were in agreement with previous studies (Wang and Stutte 1992; Escobar-Gutiérrez et al. 1998; Li and Li 2005; Meyer et al. 2004). The stress-induced changes in sorbitol content were related to the intensity and duration of the experimental drought stress. Sorbitol accumulated first in roots after a 2-h stress treatment, which was earlier than the response seen in leaves and phloem. This result indicates that micropropagated apple roots may be the most sensitive organ to drought stress, and adjust cell turgor by increasing sorbitol content.

A study in peach fruits (Escobar-Gutiérrez et al. 1998) has shown that a linear increase in S6PDH activity with drought stress correlates with a significant accumulation of sorbitol in phloem sap of severely stressed plants. The results in peach indicate that an up-regulation in sorbitol synthesis allowed sorbitol to accumulate in phloem. The activity of aldose-6-phosphate reductase (A6PR), which is the key enzyme in sorbitol synthesis, increased in expanded leaves of micropropagated apple plants subjected to different intensities of drought stress. The opposite was true for sorbitol dehydrogenase (SDH) (Bianco et al. 2000). The increased activity of A6PR and the decrease of SDH are important factors that contribute to the accumulation of sorbitol (Li and Li 2005).

Many physiological processes in plants are influenced by stress (Gao et al. 2010; Xu et al. 2001; Zhang et al. 2011). Plant responses to drought are complex, involving both coordinated gene expression and the integration of multiple biochemical pathways (Stolf-Moreira et al. 2011). The transport of sorbitol is a key factor that may be attributed to sorbitol accumulation under drought stress.

RT-qPCR results showed that mRNA levels of *MdSOT3*, *MdSOT4* and *MdSOT5* in roots increased rapidly as soon as the micropropagated apple plants were subjected to SES, and these elevated expression levels persisted until the end of treatment (Figs. 3a, 4a and 5a). This result could be explained by certain characteristics of A6PR. The activity of A6PR was not detectable in roots of micropropagated apple plants, suggesting that sorbitol could not be synthetized in roots (Li and Li 2007b). The sorbitol change in roots can also be attributed to the rapid increase in sorbitol transporter genes. Increased sorbitol transporters load more sorbitol onto phloem and roots. It is likely that apple plants adapt their carbohydrate production capability in order to resist drought stress and this is mediated by the enhancement of sorbitol transporter expression. This result was similar to the mRNA expression change of *PmPLT1* and *PmPLT2* in Plantago under salt stress (Pommerrenig et al. 2007).

The general change in sorbitol transporter expression levels of *MdSOT3* and *MdSOT5* in all organs and *MdSOT4* in roots under drought stress is consistent with the fact that sorbitol content increased between 0 and 12 h. This shows that the expression of transport genes was increased with continued drought stress time and degree of drought stress.

A study on *Mesembryanthemum crystallinum* shows that the expression of the putative myo-inositol transporter, *Mitr1*, increased in all organs, but most dramatically in roots under salt stress (Chauhan et al. 2000). Conversely, the expression of *AtSUC3* (a sucrose transporter from Arabidopsis sieve elements and sink tissues) was strongly induced upon wounding of Arabidopsis tissue (Meyer et al. 2004). In Figs. 3 and 5, the high expression levels maintained during the entire treatment process coincides with the responses of the monosaccharide transporter *STP4* in Arabidopsis (Gao et al. 2005).

Regulated expression of *MdSOT4* differs from that of *MdSOT3* and *MdSOT5* in phloem and leaves. This observation may be accounted for by the variable activity of the three sorbitol transporters. In yeast, MdSOT3- or

MdSOT5-dependent sorbitol uptake is 62 or 17 times higher compared to controls, respectively. Transport activity of MdSOT4, however, is only 1.5 times higher than in controls (Ehneβ and Roitsch 1997). The strong up-regulation of *MdSOT3* and *MdSOT5* is important for increasing the sorbitol content distribution in different organs. Therefore, the resistant ability of apple plants is improved under drought stress. A similar response also occurs in studies of the tonoplast monosaccharide transporters (*TMTs*). *TMT1* and *TMT2* expression is induced by drought, salt, cold treatment and sugar. Environment stress and sugar do not result in the accumulation of *TMT3* mRNA (Alexandra et al. 2006).

In conclusion, the mRNA levels of sorbitol transporters were influenced by MIS and SES. All three transporter genes were induced by drought stress with the exception of *MdSOT4* in phloem and leaves. *MdSOT3* and *MdSOT5* play an important role in resisting drought stress and more research is needed to characterize the function of *MdSOT4* under drought stress.

Acknowledgements We gratefully acknowledge the state key laboratory for agro-biotechnology support of RT-qPCR, the 211 State Key Laboratory of College of Agronomy and Biotechnology. We are also thankful for the support of the National Natural Science Foundation Projects (No.30871696), and National Department Public Benefit Research Foundation (No. 201003021).

References

Alexandra W, Oliver T, Ingmar F, Christian L, Joachim T, Stefan M, Ulrike S, Martinoia E, Ekkehard NH (2006) Molecular identification and physiological characterization of a novel monosaccharide transporter from Arabidopsis involved in vacuolar sugar transport. Plant Cell 18:3476–3490

Bianco RL, Rieger M, Sung SS (2000) Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. Physiol Plant 108:71–78

Chauhan S, Forsthoefel N, Ran Y, Quigley F, Nelson DE, Bohnert HJ (2000) Na^+/myoinositol symporters and Na^+/H^+-antiport in *Mesembryanthemum crystallinum*. Plant J 24:511–522

Cui S, Chen GL, Nii N (2003) Effects of water stress on sorbitol production and anatomical changes in the nuclei of leaves and root cells young loquat trees. J Am Soc Hortic Sci 72:359–365

Cui S, Sadayoshi K, Ogawa Y, Nii N (2004) Effects of water stress on sorbitol content in leaves and roots, anatomical changes in cell nuclei, and starch accumulation in leaves of young peach trees. J Am Soc Hortic Sci 73:25–30

Deguchi M, Saeki H, Ohkawa W, Kanahama K, Kanayama Y (2002) Effects of low temperature in sorbitol biosynthesis in peach leaves. J Am Soc Hortic Sci 71:446–448

Ehneβ R, Roitsch T (1997) Coordinated induction of mRNA for extracellular invertase and a glucose transporter in *Chenopodium rubrum* by cytokinins. Plant J 11:539–548

Escobar-Gutiérrez AJ, Zipperlin B, Carbonne F, Moing A, Gaudillère JP (1998) Photosynthesis, carbon partitioning and metabolite content during drought stress in peach seedlings. Aust J Plant Physiol 25:197–205

Fan RC, Peng CC, Xu YH, Wang XF, Li Y, Shang Y, Du SY, Zhao R, Zhang XY, Zhang DP (2009) Apple sucrose transporter SUT1 and sorbitol transporter SOT6 interact with cytochrome *b5* to regulate their affinity for substrate sugars. Plant Physiol 150:1880–1901

Fillion L, Ageorges A, Picaud S, Coutos-Thevenot P, Lemoine R, Romieu C, Delrot S (1999) Cloning and expression of a hexose transporter gene expressed during the ripening of grape berry. Plant Physiol 120:1083–1093

Gao ZF, Jayanty S, Beaudry R, Loescher W (2005) Sorbitol transporter expression in apple sink tissues: implications for fruit sugar accumulation and water-core development. J Am Soc Hortic Sci 130:261–268

Gao Y, Zhao Y, Li TT, Ren CX, Liu Y, Wang ML (2010) Cloning and characterization of a G protein β subunit gene responsive to plant hormones and abiotic stresses in *Brassica napus*. Plant Mol Biol Rep 28:450–459

Gao ZF, Maurousset L, Lemoine R, Yoo SD, Van Nocker S, Loescher W (2003) Cloning, expression, and characterization of sorbitol transporters from developing sour cherry fruit and leaf sink tissues. Plant Physiol 131:1566–1575

Hsiao TC (1973) Plant response to water stress. Annu Rev Plant Physiol 69:691–696

Hu L, Lu H, Liu Q, Chen X, Jiang X (2005) Overexpression of *mtlD* gene in transgenic *Ppulus tomentosa* improves salt tolerance through accumulation of mannitol. Tree Physiol 25:1273–1281

Kühn C, Barker L, Bürkle L, Frommer WB (1999) Update on sucrose transport in higher plants. J Exp Bot 50:935–953

Kühn C, Barker L, Burkle L, Frommer WB (1997) Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science 275:1298–1300

Lalonde S, Tegeder M, Throne-Holst M, Frommer WB, Patrick JW (2003) Phloem loading and unloading of sugars and amino acids. Plant Cell Environ 26:37–56

Li TH, Li SH (2007a) Leaf responses of micropropagated apple plants to water stress: changes in endogenous hormones and their influence on carbohydrate metabolism. Agric Sci China 6:58–67

Li TH, Li SH (2007b) Enzymatic regulation of sorbitol metabolism in micropropagated apple plants in response to water stress. Eur J Horticul Sci 72:12–19

Li TH, Li SH (2005) Leaf responses of micropropagated apple plants to water stress: nonstructural carbohydrate composition and regulatory role of metabolic enzymes. Tree Physiol 25:495–504

Loescher WH, Everard JD (1996) Sugar alcohol metabolism in sinks and sources. In: Zamski E, Schaffer AA (eds) Photoassimilate distribution in plant and crops: source-sink relationships. Marcel Dekker, New York, pp 185–207

Maqbool A, Zahur M, Husnain T, Riazuddin S (2009) GUSP1 and GUSP2, two drought-responsive genes in *Gossypium arboreum* have homology to universal stress proteins. Plant Mol Biol Rep 27:109–114

Meyer S, Lauterbach C, Niedermeier M, Barth I, Sjolund RD, Sauer N (2004) Wounding enhances expression of *AtSUC3*, a sucrose transporter from Arabidopsis sieve elements and sink tissues. Plant Physiol 134:684–693

Noiraud N, Maurousset L, Lemoine R (2001) Transport of polyols in higher plants. Plant Physiol Biochem 39:717–728

Pommerrenig B, Papini-Terzi FS, Sauer N (2007) Differential regulation of sorbitol and sucrose loading into the phloem of *Plantago major* in response to salt stress. Plant Physiol 144:1029–1038

Prabu G, Kawar PG, Pagariya MC, Prasad DT (2011) Identification of water deficit stress upregulated genes in sugarcane. Plant Mol Biol Rep 29:291–304

Rejskova A, Patkova L, Stodulkova E, Lipavska H (2007) The effect of abiotic stresses on carbohydrate status of olive shoots (*Olea europaea* L.) under in vitro conditions. J Plant Physiol 164:174–184

Soria-Guerra RE, Rosales-Mendoza S, Gasic K, Wisniewski ME, Band M, Korban SS (2011) Gene expression is highly regulated in early developing fruit of apple. Plant Mol Biol Rep. doi:10.1007/s11105-011-0300-y

Stadler R, Buttner M, Ache P, Hedrich R, Ivashikina N, Melzer M, Shearson SM, Smith SM, Sauer N (2003) Diurnal and light-regulated expression of AtSTP1 in guard cells of Arabidopsis. Plant Physiol 133:528–537

Stolf-Moreira R, Lemos EGM, Carareto-Alves L (2011) Transcriptional profiles of roots of different soybean genotypes subjected to drought stress. Plant Mol Biol Rep 29:19–34

Stoop JMH, Williamson JD, Pharr DM (1996) Mannitol metabolism in plants: a method for coping with stress. Trends Plant Sci 1:139–144

Su ZJ, Li XH, Hao ZF, Xie CX, Li MS, Weng JF, Zhang DG, Liang XL, Wang ZG, Gao JL, Zhang SH (2010) Association analysis of the *nced* and *rab28* genes with phenotypic traits under water stress in Maize. Plant Mol Biol Rep. doi:10.1007/s11105-010-0279-9

Truernit E, Schmid J, Epple P, Illig J, Sauer N (1996) The sink-specific and stress-regulated Arabidopsis *STP4* gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge. Plant Cell 8:2169–2182

Wan CY, Wilkins TA (1994) A modified hot borate method significantly enhances the yield of high quality RNA from cotton (*Gossypium hirsutum* L). Anal Biochem 223:7–12

Wang Z, Stutte GW (1992) The role of carbohydrates in active osmotic adjustment in apple under water stress. J Am Soc Hortic Sci 117:816–823

Wang SC, Liang D, Shi SG, Ma FW, Shu HR, Wang RC (2011) Isolation and characterization of a novel drought responsive gene encoding a glycine-rich RNA-binding protein in *Malus prunifolia* (Willd.) Borkh. Plant Mol Biol Rep 29:125–134

Watari J, Kobae Y, Yamaki S, Yamada K, Toyofuku K, Tabuchi T, Shiratake K (2004) Identification of sorbitol transporters expressed in the phloem of apple source leaves. Plant Cell Physiol 45:1032–1041

Xu CX, Zheng L, Gao CQ, Wang C, Liu GF, Jiang J, Wang YC (2001) Ovexpression of a vacuolar H^+-ATPase c subunit gene mediates physiological changes leading to enhanced salt tolerance in transgenic tobacco. Plant Mol Biol Rep 29:424–430

Yamasaki S, Dillenburg LR (1999) Measurements of leaf relative water content in *araucaria angustifolia*. Rev Bras Fisiol Veg 11:69–75

Zhang X, Zhen JB, Li ZH, Kang DM, Yang YM, Kong J, Hua JP (2011) Expression profile of early responsive genes under salt stress in upland cotton (*Gossypium hirsutum* L.). Plant Mol Biol Rep. doi:10.1007/s11105-010-0269-y

Yang GL, Zhou RC, Tang T, Chen XS, Ouyang JH, He L, Li WJ, Chen SF, Guo MM, Zhong CR, Shi SH (2010) Gene expression profiles in response to salt stress in *Hibiscus Tiliaceus*. Plant Mol Biol Rep. doi:10.1007/s11105-010-0267-0