RESEARCH NOTE

Diverse expression of sucrose transporter gene family in *Zea mays*

B. USHA[1]*, D. BORDOLOI[1] and AJAY PARIDA[2]

[1]*Department of Genetic Engineering, Sri Ramasamy Memorial University, Kattankulathur 603 203, India*
[2]*Plant Molecular Biology Laboratory, M. S. Swaminathan Research Foundation, Taramani, Chennai 600 113, India*

[Usha B., Bordoloi D. and Parida A. 2015 Diverse expression of sucrose transporter gene family in *Zea mays*. *J. Genet.* **94**, 151–154]

Zea mays, a water use efficient crop with sugar-rich tissues, has a very good potential as an alternative feedstock for ethanol production. Sucrose represents one of the major carbohydrate transport forms of photosynthetically assimilated carbon in plants. Hence, it is crucial to understand the molecular mechanism of sucrose transportation in *Z. mays*. In this study, we identified four sucrose transporter genes (*ZmSUT1, ZmSUT2, ZmSUT4 and ZmSUT5*) through extensive search at maize genome database and NCBI database with rice *OsSUTs* as query sequence. Spatial expression studies using reverse transcription polymerase chain reaction (PCR) showed that *ZmSUT1* and *ZmSUT2* are abundant in leaves than in roots while *ZmSUT4* did not show any alteration in expression level. *ZmSUT5* transcript level was higher in roots. Phosphate starvation had led to an increase in the level of *ZmSUT1* and *ZmSUT2* transcripts without affecting the expression of *ZmSUT4*. Increase in sucrose level has been observed as an early response to phosphate starvation in many plants. Thus, this study demonstrates that *ZmSUT1* and *ZmSUT2* being upregulated in response to Pi stress, may be involved in the transport of sucrose in maize while *ZmSUT4* remains a house-keeping gene.

Sucrose transporters (SUTs) are a family of proteins that transport sucrose from its site of synthesis in source tissues (leaves) to sink tissues (roots, fruits, etc). SUTs acquire energy from H^+/ATPases and follow apoplastic pathway to transport sucrose from the photosynthetic cells to phloem tissues (Tarpley and Vietor 2007). SUTs are hydrophobic proteins that form a pore with 12 transmembrane spanning domains and are located in the plasma membrane or tonoplast of storage cells (Sauer 2007).

Based on sequence homology and biochemical activity, SUTs were previously divided into three types: type I, type II and type III (Sivitz *et al.* 2005). Type I composed exclusively of dicot sequences but types II and III contained both monocot and dicot sequences. With the recent increase in the number of *SUT* sequences, phylogenetic analysis of SUTs from different plants has divided them into four clades and five groups. Groups 1 and 5 (formerly type II) consist entirely of monocot SUTs, group 2 (formerly type I) consist only dicot SUTs, and both groups 3 (formerly type II) and 4 (formerly type III) consist both monocot and dicot SUTs (Braun and Slewinski 2009).

Despite the availability of whole genome sequence data for various monocot species, *SUT* gene family has been identified only from *Oryza sativa* (Aoki *et al.* 2003) and *Sorghum bicolor* (Qazi *et al.* 2012). Transcript analysis revealed a higher expression of *OsSUT1, OsSUT3* and *OsSUT5* in germinating seeds, source leaf sheaths, panicles and developing grains and at lower levels in roots and germinating seeds. However, *OsSUT2* is expressed at low levels in the tissues analysed which is similar to that found for barley *HvSUT2* (Weschke *et al.* 2000). *SUTs* show differential expression in different plants species, *OsSUT1* and *HvSUT1* show low transcript levels in leaf blades of rice and barley, while *ZmSUT1* shows strong expression in maize leaves (Hirose *et al.* 1997; Aoki *et al.* 1999; Weschke *et al.* 2000). Such variation at the species level suggests the possibility of diverse roles SUTs possess in source and sink tissues.

SUT genes reveal differential expression not only by organ or tissue specificity but also under different developmental cues, nutrient deficiency, stress and environmental conditions (Shiratake 2007). Phosphate deficiency is one of the major problems leading to delayed maturity, reduced quality of forage, fruit, vegetable and grain crops, and decreased disease resistance. Sugar signalling is the immediate response of Pi starvation in a plant which follows increased sucrose biosynthesis in source tissues (Karthikeyan *et al.* 2007). Sucrose is loaded to the phloem and directed to roots for increase in their size thus allowing uptake of available phosphorus. Sugar signalling cascades also alter the expression of inorganic Pi transporters and acid phosphatases for the release of phosphorus from the soil (Hammond and White 2008).

*For correspondence. E-mail: sundaram.usha@gmail.com.

Keywords. sucrose transporters; maize; phosphate starvation.

Although, there is strong evidence for increase in sugar levels in response to Pi starvation there are no reports available on the function of SUTs under reduced phosphate levels.

Till date, only *SUT1*gene (*ZmSUT1*) has been identified and characterized for *Z. mays* (Slewinski *et al.* 2009). In this study, we have identified a family of four *SUT* genes from NCBI and genome database of maize. The expression pattern of these genes was studied in leaves and roots using semiquantitative PCR. We also examined if these genes are upregulated by elevated sucrose caused due to Pi starvation. The possible roles of different SUTs in *Z. mays* are discussed.

The cDNA sequences coding for SUTs for maize along with their chromosomal locations were retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/). Phylogenetic tree was constructed for *SUT* genes from monocot plant species using MEGA ver. 5 (Tamura *et al.* 2011). Motif analysis was performed for amino acid sequences of all *ZmSUT* genes in *Z. mays* to confirm if they possessed H^+/sucrose symporter activity for distribution of sucrose in the plant.

Maize seeds *(Z. mays* variety GANGA 5), provided by IARI, Delhi, India were thoroughly cleaned with water and allowed to germinate in petri dish with moist cotton gauze for three days. The germinated seeds were then transferred to soil and allowed to grow in green house at 28°C with 14 h light / 10 h dark cycles. Leaves and roots were harvested from 14 day-old seedlings for total RNA isolation and expression analysis. For phosphate starvation, 14 day-old plants from green house were transferred to glass flasks containing half-strength MS nutrient liquid medium without phosphorus (substituting K_2SO_4 for KH_2PO_4). RNA was isolated from leaves and roots of treated and control plants after five days.

Total RNA was isolated from 200 mg of leaves and roots using TRIzol reagent (Invitrogen, Carlsbad, USA) according to manufacturer's instructions and treated with DNase. RNA obtained after the removal of residual enzyme was dissolved in 30 μL of nuclease free water and stored at −80°C until further use. First strand cDNA was synthesized from each RNA sample using the cDNA kit (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, USA) with the gene specific primer for *ZmSUT1, ZmSUT2, ZmSUT4* and *ZmSUT5* designed at the 3′-UTR regions according to manufacturer's instructions. Second strand synthesis was done with forward and reverse primers designed at 3′-UTR. (PCR conditions were 5 min initial denaturation at 94°C; 35 PCR cycles for 30 s at 94°C, 25 s at 54°C, 25 s at 72°C; a final extension of 3 min at 72°C). RNA from leaf and root tissues of normal and treated plants were equalized using endogenous *β-actin* gene. Primer sequence and accession number of the genes are given in table 1.

In this study, a family of six *SUT* cDNA sequences were retrieved from NCBI and maize genome database. Multiple sequence alignment of deduced amino acid sequences of these genes revealed two redundant sequences, and hence four sequences were considered for further studies.

Table 1. Primers of *ZmSUT* genes used for semiquantitative RT-PCR analysis.

Gene	Accession no.	Primer sequence (5′–3′)
ZmSUT1	NM_00111137	F: CATGCATGTCATGTGTGTGCTT
		R: CCTTAACTGACCTACTCGCT
ZmSUT2	NM_001144014	F: TGCCATGTAAAACATCACAC
		R: CACCTTCGCATCAACCATCC
ZmSUT4	AY581895	F: CTATTATCTATCTCCAGGCAGG
		R: TCAAACACCTCCTTAATCGGGT
ZmSUT5	EU961016	F: CCATTACAGGCATCATCAAGTC
		R: CATACATGCATACGTGCGTACA
ZmACTB	NM_001155179	F: GACATGGTACGTCAGGCGTT
		R: CTCTGAGGCAACACGTTACA

Phylogenetic tree constructed on the basis of the deduced amino acid sequences of monocot SUTs revealed a close relationship of ZmSUTs with *S. bicolor* (figure 1). However, to maintain consistency in denoting cereal *SUT* genes, four *Z. mays SUT*s were named *ZmSUT1*, *ZmSUT2*, *ZmSUT4* and *ZmSUT5* based on their homology to rice *SUT*s (Aoki *et al*. 2003). The four *ZmSUT*s can be categorized into group 1, group 4, group 3 and group 5, respectively according to Braun and Slewinski (2009).

Analysis of cDNA and gene sequences show the presence of 13, 6, 13 and 9 introns, respectively for *ZmSUT1*, *ZmSUT2*, *ZmSUT4* and *ZmSUT5*. Comparison of the gene structure of five members of *OsSUT*s with *ZmSUT*s reflect similarities in the intron organization (Aoki *et al.* 2003). This shows that the position and intron number are highly conserved across plant species and can be partly used to classify the genes. Besides, introns also contribute in part as exons to the level and pattern of gene expression. For instance, introns 3 and 2 of *LeSUT1* are responsible for the expression in trichomes and guard cells, respectively (Weise *et al.* 2008). In another study, removal of leader introns in sucrose synthase 3 (*Sus3*) gene in arabidopsis led to altered expression (Fu *et al*. 1995).

Transmembrane and topology prediction of ZmSUTs showed presence of 12 membrane spanning alpha helix with the central loop, and the N-,C-termini present in the cytoplasm as seen in other plant SUTs (Lemoine 2000). Multiple sequence alignment of the deduced amino acid sequences of the four *ZmSUT*s revealed the presence of highly conserved histidine residue and other motifs in the extra cellular loop that bind and transport sucrose (Lu and Bush 1998) (figure 2). Conservation of these motifs across plant species suggests that all *ZmSUT*s code for functional *SUT* proteins.

Transcript expression analysis of *ZmSUT* genes in leaves and roots by RT-PCR revealed that all four genes were expressed in both leaf and root tissues (figure 3). Expression of *ZmSUT1* and *ZmSUT2* are found to be more pronounced in leaves as compared to roots. *ZmSUT4* showed consistent expression in both the tissues while *ZmSUT5* was higher in roots than leaves. A study conducted by Aoki *et al.* (1999), also showed abundance in *ZmSUT1* transcript in source and

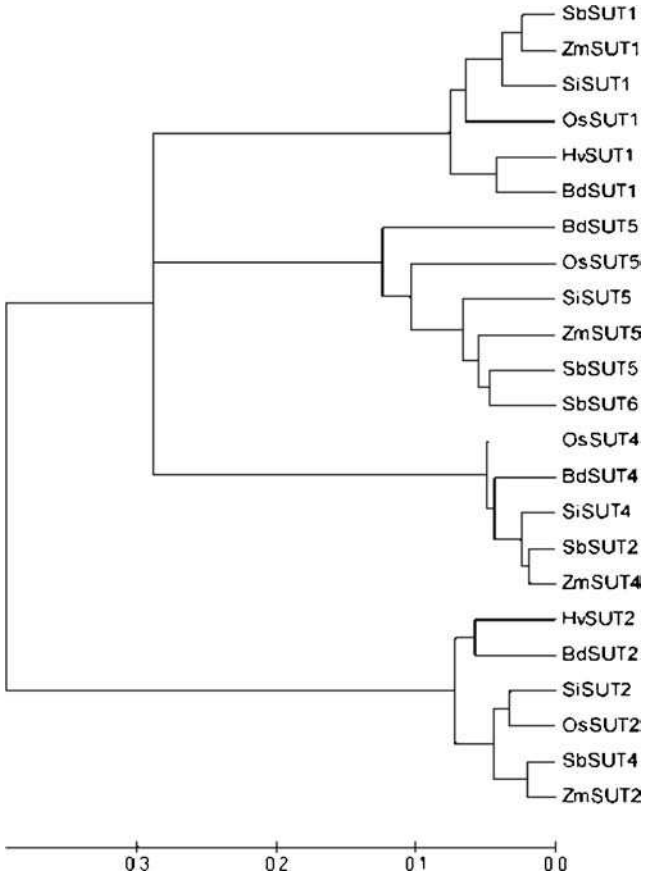

Figure 1. Phylogenetic tree of monocot SUTs based on deduced amino acid sequences. The accession numbers of SUTs from *O. sativa* OsSUT1 AAF90181, OsSUT2 BAC67163, OsSUT4 BAC67164, OsSUT5 BAC67165; from *Z. mays* ZmSUT1 BAA83 501, ZmSUT2 NP_001137486, ZmSUT4 AAS91375, ZmSUT5 ACG33134; from *Brachypodium distachyon* BdSUT1 XP_00 3558709, BdSUT2 XP_003577278, BdSUT4 XP_003570488, BdSUT5 XP_003575214; from *Hordeum vulgare* HvSUT1 CAJ 20123, HvSUT2 CAB75881; from *S. bicolor* SbSUT1 ACY69230, SbSUT2 XP_002453083, SbSUT4 ACX71839, SbSUT5 XP_ 002454058, SbSUT6 XP_002445925; from *Setaria italica* SiSUT1 XP_004985542, SiSUT2 XP_004963349, SiSUT4 XP_004954470, SiSUT5 XP_004952871.

sink tissues. Spatial expression pattern of rice SUTs revealed the abundance of *OsSUT1, OsSUT3, OsSUT4* and *OsSUT5* in source and sink leaf tissues as compared to root tissues (Aoki *et al.* 2003). Transcription of *OsSUT2* was unaltered in all the tissues analysed indicating a constitutive expression similar to *ZmSUT4* in maize. The differential expression of *ZmSUT* genes suggest their diverse role in source and sink tissues of maize.

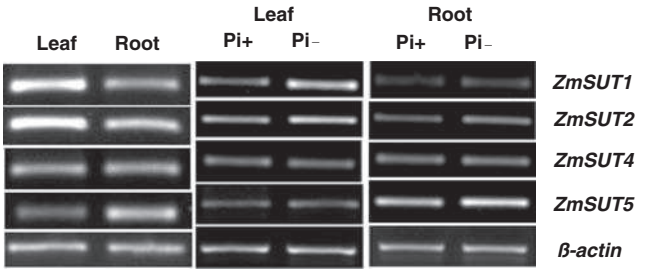

Figure 3. Semiquantitative RT-PCR analysis of *ZmSUT* genes under normal and phosphate starvation conditions. RT-PCR was performed using total RNA isolated from leaf and root tissues of 14 day-old maize seedlings grown in soil (for normal condition) and in half-strength MS basal medium with or without phosphate for Pi starvation condition.

To examine whether these transporters are upregulated by increased sugar levels, we declined the plants of phosphorus to increase internal sucrose concentration. Several studies state that phosphate starvation leads to increased sucrose biosynthesis as an early response which further causes alteration of several genes involved in overcoming phosphate unavailability (Hammond and White 2008). When maize plants were deprived of phosphorus, the transcript levels of *ZmSUT1* and *ZmSUT2* increased in leaves while no significant changes were observed in roots. The level of *ZmSUT4* mRNA was not altered under Pi starvation, thus showing constitutive expression in both leaves and roots. *ZmSUT5* was slightly upregulated in roots after Pi deprivation, while the levels remained unchanged in the leaf tissues (figure 3). These alterations are caused by enhanced sucrose levels in the plant tissues as a result of phosphorus starvation.

The rate of transcription of SUTs in other plants increased with higher sucrose levels as shown in rice. Upon supply of sucrose to rice plants, expression of *OsSUT1* increased in the leaves (Ishimaru *et al.* 2001). Similar results have also been shown for *ZmSUT1*, where the gene expression increased with higher sucrose concentration in the leaves of *Z. mays* (Aoki *et al.* 1999). However, till date, there are no reports on the impact of Pi starvation on SUTs. This study illustrates the alteration in the levels of *SUT* genes in *Z. mays* due to Pi starvation which leads to enhanced sucrose biosynthesis. We hypothesize that *ZmSUT1* and *ZmSUT2* that are upregulated by sucrose, are involved in the transport of sucrose in the source tissues. Further studies are underway to explore the role of these transporters. An in-depth knowledge on the gene expression pattern will enable the creation of crop plants to cope with Pi stress and also to increase the sugar

```
ZmSUT1   RLILAGMVAGGVQYGWALQLSLLTPYVQTLGLSHALTSFMWLCGPIAGLVVQPLVGLYSD
ZmSUT2   KLLRAASVACGVQFGWALQLSLLTPYVQELGIPHAFASLVWLCGPLSGLLVQPLVGHLSD
ZmSUT4   KLVLACMVAAGVQFGWALQLSLLTPYIQTLGIDHAMASFIWLCGPITGFVVQPCVGVWSD
ZmSUT5   RLFLACMVSGGIQYGWALQLSLLSPYSQTLGISHSYVSLTWICGPIAGFVVQPIVGYYSD
```

Figure 2. Putative transmembrane domains of the *SUT* peptides from *Z. mays* are highlighted. The functionally important and conserved histidine residue is also highlighted.

levels in *Z. mays* thus increasing its use as a feedstock for ethanol production.

Acknowledgement

The authors thank Dr G. Ganesh, M. S. Swaminathan Research Foundation, Chennai, India for his assistance in the preparation of this manuscript.

References

Aoki N., Hirose T., Takahashi S., Ono K., Ishimaru K. and Ohsugi R. 1999 Molecular cloning and expression analysis of a gene for a sucrose transporter in maize (*Zea mays* L.) *Plant Cell Physiol.* **40**, 1072–1078.

Aoki N., Hirose T., Scofield G. N., Whitfeld P. R. and Furbank R. T. 2003 The sucrose transporter gene family in rice. *Plant Cell Physiol.* **44**, 223–232.

Braun D. M. and Slewinski T. L. 2009 Genetic control of carbon partitioning in grasses: Roles of sucrose transporters and tie-dyed loci in phloem loading. *Plant Physiol.* **149**, 71–81.

Fu H., Kim S. Y. and Park W. D. 1995 A potato Sus3 sucrose synthase gene contains a context-dependent 30 element and a leader intron with both positive and negative tissue-specific effects. *Plant Cell* **7**, 1395–1403.

Hammond J. P. and White P. J. 2008 Sucrose transport in phloem: integrating root responses to phosphate starvation. *J. Exp. Bot.* **59**, 93–109.

Hirose T., Imaizumi N., Scofield G. N., Furbank R. T. and Ohsugi R. 1997 cDNA cloning and tissue-specific expression of a gene for sucrose transporter from rice (*Oryza sativa* L.) *Plant Cell Physiol.* **38**, 1389–1396.

Ishimaru K., Hirose T., Aoki N., Takahashi S., Ono K., Yamamoto S. *et al.* 2001 Antisense expression of rice sucrose transporter *OsSUT1* in rice (*Oryza sativa* L.) *Plant Cell Physiol.* **42**, 1181–1185.

Karthikeyan A. S., Varadarajan D. K., Jain A., Held M. A., Carpita N. C. and Raghothama K. G. 2007 Phosphate starvation responses are mediated by sugar signalling in *Arabidopsis*. *Planta* **225**, 907–918.

Lemoine R. 2000 Sucrose transporters in plants: update on function and structure. *Biochem. Biophys. Acta* **1465**, 246–262.

Lu J. M. Y. and Bush D. R. 1998 His-65 in the proton-sucrose symporter is an essential amino acid whose modification with site-directed mutagenesis increases transport activity. *Proc. Natl. Acad. Sci. USA* **95**, 9025–9030.

Qazi H. A., Paranjpe S. and Bhargava S. 2012 Stem sugar accumulation in sweet sorghum-activity and expression of sucrose metabolizing enzymes and sucrose transporters. *J. Plant Physiol.* **169**, 605–613.

Sauer N. 2007 Molecular physiology of higher plant sucrose transporters. *FEBS Lett.* **581**, 2309–2317.

Shiratake K. 2007 Genetics of Sucrose Transporter in Plants. *Global Science Books* **1**, 73–78.

Sivitz A. B., Reinders A. and Ward J. M. 2005 Analysis of the transport activity of barley sucrose transporter HvSUT1. *Plant Cell Physiol.* **46**, 1666–1673.

Slewinski T. L., Meeley R. and Braun D. M. 2009 *Sucrose transporter1* functions in phloem loading in maize leaves. *J. Exp. Bot.* **60**, 881–892.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. 2011 MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Tarpley L. and Vietor D. M. 2007 Compartmentation of sucrose during radial transfer in mature sorghum clum. *BMC Plant Biol.* **7**, 33.

Weise A., Barker L., Kühn C., Lalonde S., Buschmann H., Frommer W. B. and Ward J. M. 2008 Introns control expression of sucrose transporter *LeSUT1* in trichomes, companion cells and in guard cells. *Plant Mol. Biol.* **68**, 251–262.

Weschke W., Panitz R., Sauer N., Wang Q., Neubohn B., Weber H. and Wobus U. 2000 Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. *Plant J.* **21**, 455–467.

Received 29 May 2014, in revised form 16 July 2014; accepted 21 October 2014
Unedited version published online: 29 October 2014
Final version published online: 4 March 2015