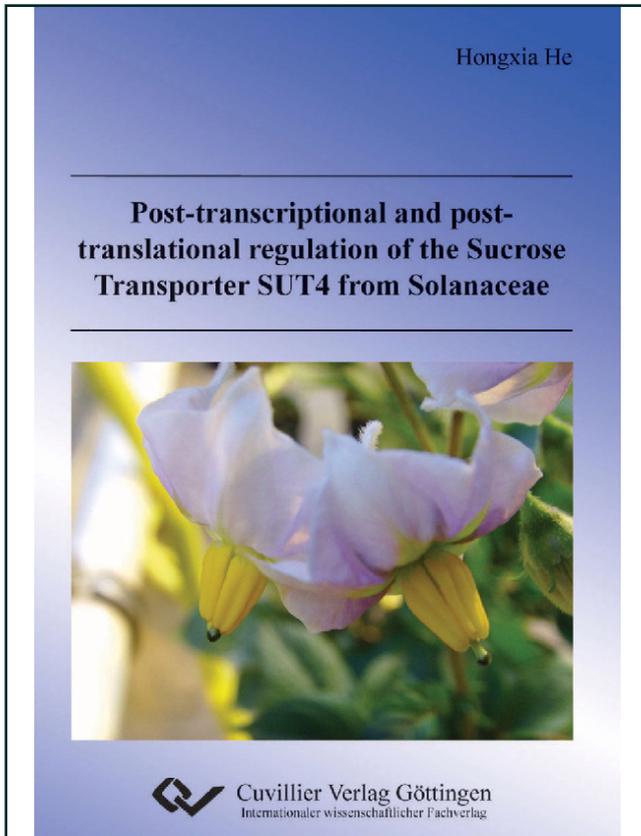Hongxia He (Autor)

Post-transcriptional and post-translational regulation of the Sucrose Transporter SUT4 from Solanaceae

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1 Introduction

1.1 Phylogenetic and physiologic analysis of sucrose transporters in plants

1.1.1 Phylogenetic analysis of sucrose transporters in plant kingdom

Sugars are the end products of photosynthesis in plants. They act as substrates for energy metabolism and biosynthesis of complex carbohydrates and provide necessary resources for the growth and development of sink tissues. In addition, sugars also serve as secondary messengers, with the ability to regulate plant growth and development in response to biotic and abiotic stresses (Hammond and White, 2008). The network of sugar signalling has the capacity to regulate the expression of various genes and to interact with other signalling pathways such as phytochrome responses and metabolic pathways (Gibson, 2004).

Sucrose, the major sugar form, is produced mainly in mature leaves (source organs) of plants. To support the growth of various heterotrophic sink tissues (developing leaves, the shoot apex, roots, and reproductive organs), sucrose distribution from source organs to respective tissues is mediated by the vascular system. The enucleate sieve elements (SEs) present in the vascular tissue of the phloem and form the conduits for long distance transport (Weise et al., 2000). Sucrose transport has been studied in a broad variety of plant species and sucrose uptake has often been described as a biphasic transport system. In angiosperms the kinetic of sucrose uptake is comprised of two components with different kinetic properties, one component represents a high affinity-low capacity carrier and the second one represents a low affinity-high capacity transporter (Kühn, 2003).

Plant Sucrose Transporters (SUTs) were first isolated via yeast complementation from both potato and spinach (Riesmeier et al., 1992; Riesmeier et al., 1993). Subsequently, genes and cDNAs encoding homologous proteins have been cloned from more than 60 plant species (Lalonde et al., 2004; Sauer, 2007). SUT proteins are predicted to be integral membrane proteins with 12 membrane spanning domains and a putative central hydrophilic loop. The 6-loop-6-structure of the 12 trans-membrane domain proteins is highly conserved among plant species and is similar to what is characteristic of the major facilitator superfamily (MFS) for several cation/substrate co-transporters (Marger and Saier, 1993). Completion of the rice, Arabidopsis and yeast genomes has allowed the identification of new sucrose transporter-like proteins and the analysis of whole gene families. Phylogenetic analysis has shown that all known sucrose transporters and sucrose transporter-like proteins fall into three clades in dicot plants (Kühn, 2003; Sauer, 2007). Kühn and Grof have carried out a new

phylogenetic analysis of sucrose transporters in monocot and dicot plants (unpublished data). In the new phylogenetic tree shown as follow, all sucrose transporters and sucrose carriers fall into five clades. The SUT1 clade contains only dicot-specific sucrose transporters (Fig. 1), which are mainly expressed in sucrose exporting source leaves (Barker et al., 2000) and are subject to regulation by plant hormones (Harms et al., 1994). Immunolocalization studies have showed that SUT1 is present at high levels in the plasma membrane of sieve elements (Kühn et al., 1997); members of the SUT2 and SUT4 clades exist in both monocot and dicot species; members of the SUT3 and SUT5 clades are monocot-specific sucrose transporters (Fig. 1).
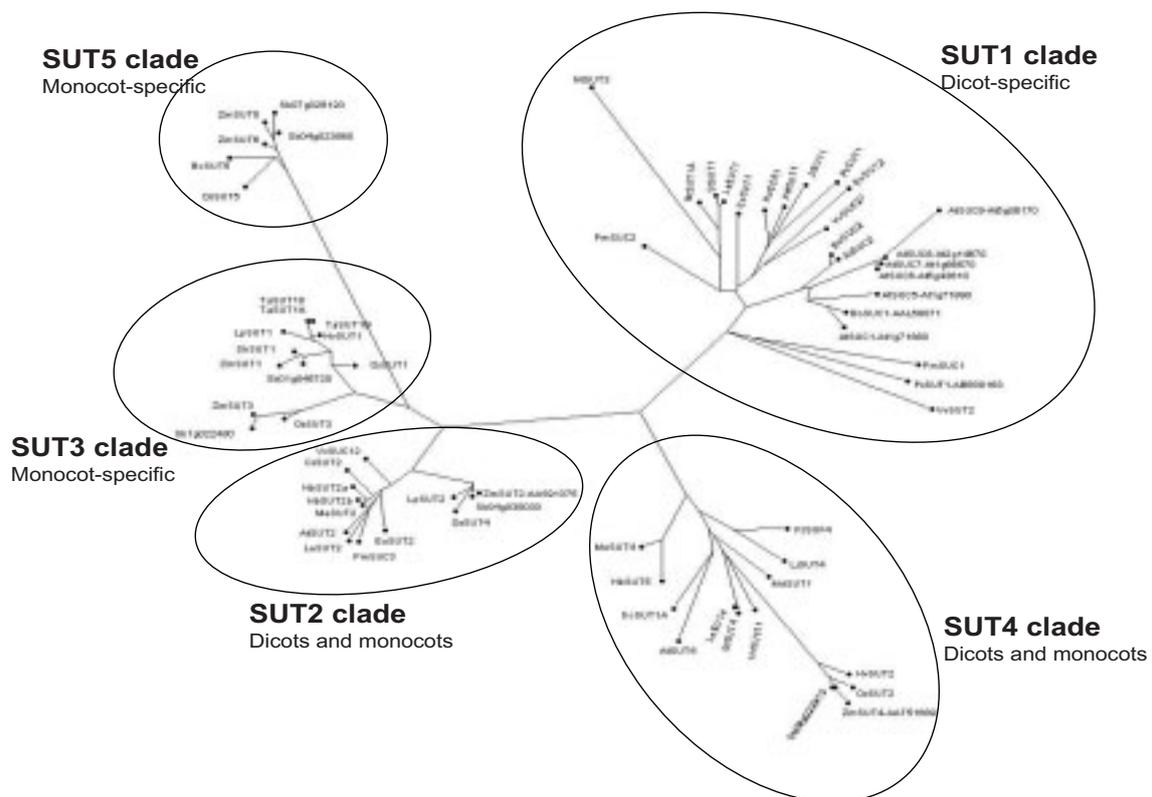

Fig. 1: A phylogenetic tree of sucrose transporters in monocotyledonous and dicotyledonous species base on the similarities of available gene sequences

The phylogenetic tree is constructed using Dendroscope (Huson et al., 2007) and is drawn by Kühn and Grof (unpublished data). Accession numbers of presented sucrose transporter are: *Arabidopsis thaliana*: AtSUC1, At1g71880; AtSUT2, At1g22710; AtSUT2, At2g02860; AtSUT4, At1g09960; AtSUC5, At1g71890; AtSUC6, At5g43610; AtSUC7, At1g66570; AtSUC8, At2g14670; AtSUC9, At5g06170. *Brassica oleracea*: BoSUC1, AAL58071; BoSUC2, AAL58072. *Bambusa oldhami*: BoSUT5, AAY43226. *Citrus sinensis*: CsSUT2, AAM29153. *Daucus carota*: DcSUT1A, CCA76367; DcSUT2, CAA76369. *Eucommia ulmoides*: AAX49396. *Hevea brasiliensis*: HbSUT2a, ABJ51934; HbSUT2b, ABJ51932; HbSUT5, ABK60189. *Hordeum vulgare*: HvSUT1, CAB75882; HvSUT2, CAB75881. Juglans regia: JrSUT1, AAU11810. *Lycopersicum esculentum* renamed *Solanum lycopersicum*: LeSUT1, AAG12987; LeSUT4, AAG09270. *Lotus japonicus*: LjSUT4, CAD61275. *Lolium pernne*: LpSUT1, EU255258; LpSUT2, ACU87542. *Malus x domestica*: MdSUT1, AAR17700. *Manihot esculenta*: MeSUT2, ABA08445; MeSUT4, ABA08443. Nicotian tabacum: NtSUT1A, CAA57727; NtSUT3, AAD34610. *Oryza sativa*: OsSUT1, AAF90181; OsSUT2, BAC67163; OsSUT3, BAB68368; OsSUT4, BAC67164; OsSUT5, BAC67165. *Plantago major*: PmSUC1, CAI59556; PmSUC2, X75764; PmSUC3, CAD58887. *Pisum sativum*: PsSUT1, AAD41024; PsSUF1, ABB30163; PsSUF4, ABB30162. *Populus tremula x Populus tremuloides*: PtSUT1-1, CAJ33718. *Ricinus communis*:

RsSCR1, CAA83436. *Saccharum hybrid*: ShSUT1, AAV41028. *Spinacea oleracea*: SoSUT1, Q03411. *Solanum tuberosum*: StSUT1, CAA48915; StSUT4, AAG25923. *Sorghum bicolor[a]*: SbSUT1, Sb01g045720; SbSUT2, Sb04g038030; SbSUT3, Sb01g022430; SbSUT4, Sb08023310; SbSUT5, Sb04g023860; SbSUT6, Sb07g028120. *Triticum aestivum*: TaSUT1A, AAM13408; TaSUT1B, AAM13409; TaSUT1D, AAM13410. *Vitus Vinifera*: VvSUC11, AAF08329; VvSUC12, AAF08330; VvSUC27, AAF08331; VvSUT2, AAL32020. *Zea mays[b]*: ZmSUT1, BAA83501; ZmSUT2, AAS91375; ZmSUT3, ACF86653; ZmSUT4, AAT51689; ZmSUT5, ACF85284; ZmSUT6, ACF85673. Accession numbers are also used as additional descriptors in the tree in those instances where confusion may arise due to variations in nomenclature.

[a]Sorghum Genome Project : www.phytozome.net

[b]Maize Genome Project: http://www.maizesequence.org/index.html. The Phylogeny website (http://www.phylogeny.fr/version2_cgi/index.cgi) was used extensively for sequence alignment and analysis(Dereeper et al., 2008). The data was converted into Newick format prior to transfer to Dendroscope (http://www-ab.informatik.uni-tuebingen.de/software/dendroscope).

1.1.2 Sucrose transporters in solanaceous plants

The Solanaceae plant family is extensively utilized by human. It is an important source of food (e.g. potato and tomato), spice (e.g. hot pepper) and medicine (e.g. datura, which produces tropane alkaloid). Some crop species such as tobacco, potato and tomato have been studied in detail with respect to carbohydrate partitioning during classical physiological methodology and molecular tools have been used for many genes involved in carbohydrate metabolism (Frommer and Sonnewald, 1995). Sucrose transporters are important for carbohydrate metabolism and are involved in sucrose phloem loading. The sucrose transporter proteins (SUT1, SUT2 and SUT4) have been demonstrated to be co-localized in sieve elements (SEs) of potato source leaves, petioles and stems (Reinders et al., 2002), whereas transcription of SUT1 is shown to take place in companion cells (CCs) (Kühn et al., 1996; Barker et al., 2000; Weise et al., 2000). These three SUT proteins belong to SUT1, SUT2 and SUT4 clades respectively and the RNA levels of them not only follow a diurnal rhythm but also oscillate in constant light (Chincinska et al., 2008). Furthermore, the mode of phloem loading and the mechanisms of SE-specific protein synthesis and protein targeting, which are described for solanaceous plants, resemble those of other plant species such as wheat and opium poppy (Bird et al., 2003; Aoki et al., 2004).

The SUT1 clade

NtSUT1, NtSUT3 (from *Nicotiana tabacum*), SlSUT1 (*Solanum lycopersicum*) and StSUT1 (from *Solanum tuberosum*) belong to the SUT1 clade (Fig. 1). StSUT1 is characterized as a high-affinity sucrose / proton co-transporter and its antisense repression leads to reduced transport activity of the protein causing extensive physiological defects in carbon partitioning and photosynthesis (Riesmeier et al., 1994; Kühn et al., 1996). The role of SUT1 as an essential protein driving sucrose against a concentration gradient into the phloem for long-distance transport is also successfully demonstrated in potato by a transgenic approach (Kühn et al., 1996). Similar effects have been observed in a seed plant (tobacco) confirming the importance of SUT1 for sucrose loading into the phloem via an apoplastic route and possibly for inter-

mesophyll transport (Bürkle et al., 1998). The phenotype of transgenic tomato plants (containing antisense transcripts of *SlSUT1*) (Hackel et al., 2006) is comparable to *SUT1* antisense phenotypes in potato and tobacco (Riesmeier et al., 1994; Kühn et al., 1996; Bürkle et al., 1998) and to the homologous *AtSUC2* insertional mutant phenotype in *Arabidopsis* (Gottwald et al., 2000). The observed phenotype of *SlSUT1* antisense plants is defective fruit development. It is probably due to an undersupply of carbohydrates to sink organs, which accumulate to high amounts in source leaves, leading to severe leaf modifications and osmotic symptoms. The development of sink organs in the *SlSUT1* antisense plants is delayed due to disturbance in supply of sugars to terminal sink organs indicating an essential function of SUT1 in phloem loading and long-distance transport of sugars. Overall, it could be said that in *solanaceous* plants, SUT1 is essential for apoplasmic phloem loading of sucrose.

The SUT2 clade

The SUT2 clade only has three known members in *Solanaceae*, SlSUT2 (from *Solanum lycopersicum*), SdSUT2 (from *Solanum demissum*) and StSUT2 (from *Solanum tuberosum*). The later two are not included in Fig. 1; the protein sequences are highly homologous to SlSUT2. The *SUT2* expression is predominantly detected in sink organs and is identified to colocalize with low and high-affinity sucrose transporters (*SUT4* and *SUT1*) in enucleate sieve elements of tomato and shares features with yeast sugar sensors (Barker et al., 2000). Most importantly, the *SUT2* gene maps on chromosome V of potato and is linked to a major quantitative trait locus (QTL) for tuber starch content and yield (Gebhardt et al., 1991). In contrast to *SlSUT1*, which is induced during the sink-to-source transition of leaves, *SUT2* is more highly expressed in sink than in source leaves and is inducible by sucrose. The SlSUT2 protein is co-localized with the low- and high-affinity sucrose transporters in SEs of tomato petioles. This indicates that multiple *SUT* mRNAs or proteins probably travel from CCs to enucleate SEs and the SUT proteins are translated in SEs (Reinders et al., 2002). Tomato plants transformed with a *SlSUT2* antisense constructs are exclusively affected in tomato fruit and seed development which is explained by three possible explanations: 1) disturbance of pollination attributed to a decreased expression of *SlSUT2* in anthers; 2) *SlSUT2* was directly involved in phloem unloading on the level of the tomato fruits or seeds; or 3) both parameters are involved in the determination of the final fruit size (Hackel et al., 2006).

The SUT4 clade

Sucrose Transporter 4 (SUT4) is originally characterized as plasma membrane localized sucrose transporters (StSUT4 and AtSUC4, HvSUT2) (Weise et al., 2000; Weschke et al., 2000). The transporter activity of SUT4 proteins has been confirmed by sucrose uptake experiments and yeast (*Saccharomyces cerevisiae*) complementation experiments with AtSUT4 (*Arabidopsis thaliana*) and StSUT4

(*Solanum tuberosum*) (Weise et al., 2000), and the orthologous HvSUT2 (*Hordeum vulgare*)(Weschke et al., 2000). The localization of StSUT4 and SlSUT4 in the plasma membrane of phloem sieve elements in potato and tomato is confirmed by immunocytochemical analysis (Weise et al., 2000; Reinders et al., 2002).

In tomato all three principal sucrose transporters, SlSUT1, SlSUT2 and SlSUT4 were described as plasma membrane localized proteins. These transporters may interact physically to adopt the phloem loading activity of solanaceous SEs to different developmental or environmental conditions. The original idea of the hypothesis was that one of these proteins, SUT2, might act as sucrose sensor (Barker et al., 2000) which can interact with a high affinity/low capacity transporter, SUT1 (Riesmeier et al., 1992), and/or with a low affinity/high capacity transporter, SUT4, and regulate the relative activities of these two proteins (Weise et al., 2000). However, Hackel *et al*., (2006) have argued that SlSUT2 might have a dual function in pollen tubes loading and in the phloem specific localization. The authors have assumed SlSUT2 serves as a sucrose transporter rather than sucrose sensor. This idea is consistent with the functional analysis of PmSUC3, which has 75% identity to SlSUT2. PmSUC3 is localized in sieve elements of the *Plantago* phloem and mediates the energy-dependent transport of sucrose and maltose (Barth et al., 2003). In fact, the analyses of potential interactions between these three sucrose transporters with the split-ubiquitin system in yeast cells support the following idea (Reinders et al., 2002; Kühn, 2003) (Fig. 2).
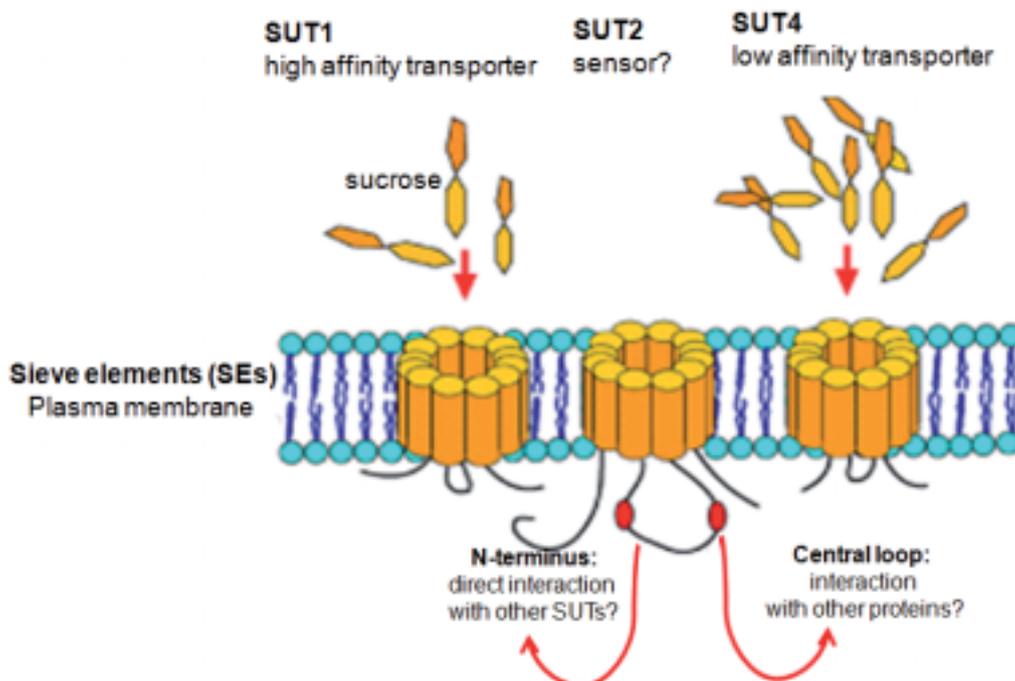

Fig. 2: Potential interaction of SUT1, SUT2 and SUT4 in solanaceous plants (Kühn, 2003)
SUT1, SUT2 and SUT4 are co-localized in the plasma membrane of sieve elements. SUT2 plays a dual function in sucrose transporting in sink organs such as pollens and fruits and a second still unknown

function in the phloem. SUT2 potentially regulates the activity of the high-affinity sucrose transporter SUT1 or the low-affinity sucrose transporter SUT4, either directly or via an indirect signaling mechanism, such as phosphorylation/dephosphorylation. Regulation can occur at the level of protein degradation, internalization of transporter or inactivation. Another possibility is regulation at the transcriptional level. Since *SUT1* and probably also the other sucrose transporter genes are most likely expressed in companion cells, this model would implicate a signal cascade affecting the transcriptional activity of the neighboring companion cells.

1.2 phloem mobile mRNAs and proteins

The phloem of higher plants is a vascular tissue and consists of living cells, enucleate sieve elements (SE) and companion cells (CC). The phloem performs a number of distinct tasks when collecting metabolites and signals from source organs (mainly mature leaves) and transporting these molecules and releasing them into sink organs (sink leaves, flowers, tubers or roots). The metabolites and signals mainly include organic substances such as sucrose and other sugars or sugar alcohols and amino acids, also a diverse range of macromolecules (proteins, RNAs and pathogens). Therefore, the phloem has great potential to facilitate inter-organ coordination and hence to promote plant growth and development (Hir et al., 2008). Plasmodesmata provide a pathway for the trafficking of proteins and RNAs. These phloem mobile signals play a major role in plant nutrition, development and communication. Proteins which move through PD are defined as non-cell-autonomous proteins (NCAPs); RNA is thought to traffic from cell to cell as ribonucleotide-protein complex (RNP). It is now well established that the local movement of NCAPs and RNPs can contribute to the establishment of cell fate and patterning in plant tissues (Lough and Lucas, 2006).

In higher plants the vascular system provides both mechanical strength and long-distance transport capacity. The phloem distributes photo-assimilates from source to sink tissues and is composed of conducting sieve elements, associated with companion cells, and non-conductive phloem parenchyma and fiber cells (Yu et al., 2007). In addition, phloem transports hormones, messenger RNAs (mRNAs), and proteins that may mediate developmental and stress responses (Citovsky and Zambryski, 2000; Lucas et al., 2001; Kehr and Buhtz, 2008). It is not only a nutrient conduit but also functions as an information highway (Lough and Lucas, 2006; Lin et al., 2009). In the previous studies, researchers have established that mRNAs molecules, small RNAs and even transfer RNAs are present in plant vascular tissues. These non-cell autonomous function as plant-unique remote-control systems in gene silencing, pathogen defense, leaf development, tuber formation, phosphate homeostasis and many other physiological processes (Ruiz-Medrano et al., 1999; Lucas et al., 2001; Yoo et al., 2004; Doering-Saad et al., 2006; Omid et al., 2007; Kehr and Buhtz, 2008; Ma et al., 2009).