Review

Sucrose assimilation and the role of sucrose transporters in plant wound response

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Plant cells are commonly exposed to a variety of injuries such as mechanical and herbivore wounding. Wounding is a continual threat to the survival of all organisms and an open wound caused by mechanical or herbivore wounding is a potential infection site for pathogens, thus expression of defense genes at the wound site is a barrier against opportunistic pathogens. Wounding in multicellular eukaryote cells result in marked changes in gene repression that contribute to cell defense and repairs. The sudden changes in cellular metabolism and additional metabolism requirements for these wounded tissues or cells can only be met by an increased utilization of exogenously supplied carbohydrate in the form of sucrose. Sucrose transporters' involvement in the import of sucrose from the apoplastic cells will be of great significance for the cellular metabolic needs, and also for energy and carbon requirements for the activation of defense responses of the adjacent injured tissues or cells.

Key words: Sucrose transporters, Plasma membrane, carbohydrate, sieve element, source-sink.

INTRODUCTION

Photosynthesis allows plants to reduce inorganic carbon, nitrogen and sulfur; generating sugar, amino acids and peptides which play major physiological roles for plants and also for heterotrophic organisms feeding on them. Specific transporters in plant cells that facilitate the uptake of sucrose, hexose and a wide range of amino acids and di- and tri-peptides are crucial for photosynthesis (Delrot et al., 2001). Membrane transport activities are essential for eukaryotic cells which invest about 12% of their genomic information in transport proteins (Tanner and Caspari, 1996). Plasma membrane transporters allow cells to fulfill part of their nutritive requirements; to indirectly participate in the control of long distance transport and assist in transducing environmental and endogenous signals (Rentsch et al., 1998).

Among all transporters, sucrose transporters play a major role because they control the long distance transport of the sieve sap via the loading and unloading process which maintains a concentration gradient between source and sink organs. It has been recognized that sucrose not only functions as substrate to sustain hetero-trophic growth of sink tissues, but is also an important signaling molecule that regulates both source and sink metabolism (Chiou and Bush, 1998; Sheen et al., 1999; Gibson, 2005).

Wounding, caused by mechanical injury or herbivorous insects, creates potential points of entry for opportunistic pathogens (Paul et al., 2000) and results in a severe environmental stress for the plant. It is therefore essential for plants to express a range of defense genes at the wound site as a response to this stress and also as a barrier against potential infection by pathogens. Many genes that contribute to tissue defense and repair are reported to be induced in *Arabidopsis* by mechanical wounding and insect herbivory (Reymond et al., 2000). Studies in plants have demonstrated that different stress related stimuli result in the same regulatory pattern of proteins and plant hormones involved in cellular metabolism and defense reactions. Defense response is tightly linked to the up-regulation of sucrose sink metabolism to satisfy the energy requirements and the activation of the cascade of defense reactions (Figure 1), thus sucrose and stress related signal transduction pathways are integrated to regulate defense reactions as well as source-sink relations (Koch, 1996; Rolland et al., 2006; Smeekens, 1998).

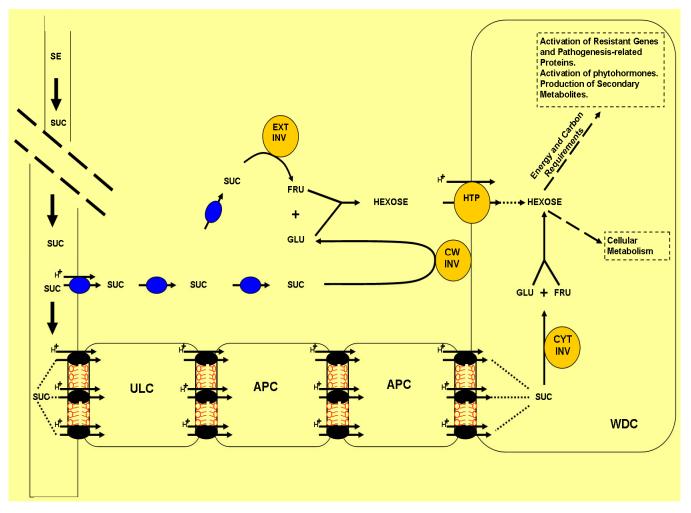*Corresponding author. E-mail: gbradley@ufh.ac.za. Tel: +27 40 602 2173. Fax: 0866288637.

Figure 1. A model for sucrose transport to a wounded cell. Sucrose is unloaded from the sieve elements (SE) of phloem into the unloading cells (ULC) by plasma membrane sucrose transporters [⬛]. Sucrose is transported via the plasma membrane from one apoplastic cell (APC) to the other, to the wounded cell (WDC). Cytosolic invertase (CYT INV) cleaves sucrose to glucose (GLU) and fructose (FRU), which are taken up for cellular metabolism and activation of defense responses. Furthermore, sucrose also leaves the sieve element via the symplastic route by sucrose efflux carriers [🔵]. Sucrose is cleaved into glucose and fructose by extracellular invertase (EXT INV) and cell wall invertase (CW INV). The resulting monosaccharides are taken up into the wounded cell by monosaccharide transporters [HTP] and used for cellular metabolism and activation of defence response.

CELLULAR LOCALIZATION OF SUCROSE TRANSPORTERS

The number of characterized sucrose transporters (SUT), also referred to as sucrose carriers (SUC) in some published reports, is increasing rapidly. In all plants so far analyzed, small families of sucrose transporter genes were found. Plant sucrose transporters belong to the major facilitator super family of transport proteins (Marger and Saier, 1993) and members differ slightly by their cellular localization and regulation of their expression. SUTs have been categorized into 4 clearly separable groups: Group 1, 2, 3, and 4, according to their cellular localization.

GROUP 1

In this group 5 proteins have been functionally charac-terized as sucrose transporters [HvSUT1, HvSUT2, OsSUT1, ShSUT1, ZmSUT1 (Sauer, 2007)]. OsSUT1 was shown to be expressed in companion cells (CCs) and both OsSUT1 and ZmSUT1 are thought to be res-ponsible for phloem loading (Scofield et al., 2007; Aoki et al., 1999). For all group1 members, additional func-tions were suggested or shown in different sink tissues in relation to sucrose import into developing grains. Thus this group represents a group of plasma membrane-loca-lized monocot sucrose transporters that catalyze primarily the uptake of sucrose into phloem and sink cells.

GROUP 2

These are plasma membrane-localized sucrose transporters from dicots. They are responsible for phloem loading or for sucrose import into different sink tissue, similar to group1 (Sauer, 2007). Gene duplication has increased the number of group 2 proteins in several plant species. Two or more group 2 genes are found in Arabidopsis, common plantain, grapevine, rape (*Brassica napus L.*) and tobacco. This allowed an optimum adaptation of parameters, such as transcriptional regulation of genes, pH-dependence and K_m values of the proteins to the specific requirements of sucrose transport into phloem of companion cells or specific sink tissues.

GROUP 3

Members of this group are easily distinguishable from other sucrose transporters. Firstly, they have a total length of roughly 600 amino acids. All members (frequently named SUC3 or SUT2- type transporters, based on the names of the first characterized transporters of this group e.g. AtSUC3 and LeSUT2) have about 15-20% more amino acids than all other sucrose transporters. Secondly, compared to other sucrose transporters they have higher K_m values for sucrose uptake. Lastly, they are localized to the sieve element (SE) in several plant species (Sauer, 2007) and are also found in sink tissues and especially in sink-leaf sieve elements, where they are proposed to be potential carriers involved in phloem unloading into the apoplast (Barth et al., 2003).

These sucrose transporters were initially published as sensors with no transport activity (Barker et al., 2000) but this does not seem to be valid any longer, because experiments have proven that they are indeed transporter proteins (Meyer et al*.,* 2000).

GROUP 4

They were originally characterized as plasma membrane localized sucrose transporters but more recently are reported to represent vacuolar sucrose transporters that are primarily expressed in sink tissues (Sauer, 2007).

Sucrose transporter sequences are highly conserved. All sucrose transporter proteins are suggested to be transmembrane proteins with 12 transmembrane helices of which N- and C- terminals are located in the cytoplasmic side of the plasma membrane as determined by hydropathy analyses (Riesmeier et al*.,* 1992; Sauer and Stolz, 1994) and immunolocalization studies with site-specific antibodies (Stolz et al*.,* 1999).

SUCROSE ASSIMILATION TO WOUNDED CELLS

The natural resistance of plants to different environmental stresses depends on a large number of inducible res-

ponses such as an increase in hydrolytic enzymes, accumulation of cell wall structural proteins, and production of antimicrobial compounds (Chisholm et al., 2006). The stress responses might take either a direct or indirect form. A direct response involves the early detection of a stress factor followed by a suitable and rapid response, while indirect defense response takes more time and is usually a result of downstream activation from the active processes.

Many of these inducible defense responses are initiated by wounding, be it mechanical or herbivory. When plants perceive stress, a response is generated that relays information along a signal transduction pathway that facilitates the initiation of appropriate physical and physiological responses. These responses require energy to activate and to maintain such induction. Injured tissues or cells are able to cope with these sudden changes in cellular metabolism and additional metabolic task caused by an increase in energy and carbon requirements that result from enhanced respiratory activities, by an increased utilization of exogenously supplied carbohydrate in the form of sucrose.

The supply of carbohydrates to sink tissue via an apoplastic pathway involves the release of the transported sucrose into the apoplast by a sucrose transporter, cleavage of dissacharides by invertase and uptake of the hexose to produce the needed energy and carbon. The 3 types of invertase i.e. extracellular, cell wall or cytosolic invertases (Figure 1) are involved in this process (Sturm, 1999). In many plant species however, sucrose leaves the symplast at some point possibly via sucrose efflux carriers and gets actively accumulated into the sieve element companion cell complex by proton coupled sucrose transporters expressed in the companion cells and sieve elements. Coupled to these sudden and urgent needs of export of photoassimilate to wounded cells (or tissues), plants tend to recognize wounded cells as sink cells, although this is artificial. Due to its stability and limited number of enzymes that catalyze its degradation, sucrose is the major form of soluble carbon and energy used in higher plants for long-distance transport. Sucrose is the exclusive and the dominant form of assimilate carbon that is used for assimilate allocation (Sauer, 2007).

Sucrose transport is a process highly regulated at the cellular level. It responds to variations in source/sink ratio which in turn depends on many internal and external parameters such as growth, development, photosynthetic activity, water status, mechanical or herbivore wounding and phytopathogen attack. This flexibility is made possible by the presence of a multigenic family whose members are expressed in different cells at different times and under different conditions. In *Arabidopsis* for example, at least 9 putative sucrose transporters are present, rice has 5 putative sucrose transporters and more have been identified in other plants. The multiple numbers of plant sucrose transporters suggests that they may be tightly regulated. The levels of expression may vary betw-

een source and sink cells and a single trans-porter may fulfill different physiological roles in different organs. The regulation of carbon partitioning between source and sink tissues in higher plants is not only important for plant growth and development, but insight into the underlying regulation mechanism is also a prerequisite in modulating assimilated partitioning during stress conditions such as wounding. There is accumulating evidence for crosstalk modulation and integration between signaling pathways responding to sucrose level and other stress-related stimuli (Koch, 1996; Chiou and Bush, 1998; Smeekens, 1998).

While sucrose is the major form of long distance transport of sugar in the phloem, monosaccharide trans-port also plays a significant role in intercellular exchange both in source and sink tissues. In sink organs, sucrose imported via the phloem is frequently hydrolyzed and the resulting monosaccharides taken up for storage or utilization. The first monosaccharide transporter (STP1) was isolated from *Arabidopsis* using the HUP1 gene from unicellular alga *chlorella kessleri* as a probe (Sauer et al., 1990; Sauer and Tanner, 1989). Further screening approaches using heterologous hybridization led to the identification of a family of 14 AtSTP genes in *Arabidopsis* (Buttner and Sauer, 2000). Despite the enormous number of monosaccharide transporters identified, only STP4 has been reported to be up-regulated in *Arabidopsis* by wounding (Truernit et al., 1996).

SUCROSE TRANSPORTER ACTIVITY DURING WOUNDING AND FUTURE PROSPECTS

Despite the dramatic and far-reaching impact of wounding stress related stimuli on agricultural yield, only few studies have focused on the regulation of sucrose transporter activities and transcriptional regulation in response to wounding, and only limited information is available on the effect of plant metabolism and source-sink relationship. Researchers have focused mainly on regulation of pathogenic related proteins, resistant genes and phytohormone regulated genes during different stress conditions, with only a few reports available on the energy requirement of such tissues or cells during stress. Sakr et al. (1997), studying the effects of mechanical stress on membrane transport of sugar in aged and freshly cut tissues of matured sugar beet (*Beta Vugaris* L.) leaves, reported that there was a vivid increase in the gene transcript of SUC1. Ageing and wounding are experimental treatments which may be related to natural events commonly encountered by plants. These include: mechanical stress due to wind, feeding by cattle or insects, attacks by phytopathogens and other agricultural practices such as grafting, pruning and mowing. From the experimental results it was clear that cutting and age- ing induce distinctive changes in the pattern of sucrose uptake in the leaf disc, therefore it was concluded that the

rise in sucrose uptake was due to a direct change in the activity of the sucrose transport system.

In another study, Meyer et al. (2004) showed that SUC3 reporter protein was reproducibly increased at all cutting sites of *Arabidopsis thaliana*. Strong SUC3 expression was found in several non-photosynthetic, symplastically isolated, rapidly growing and metabolically active cells and tissues such as: guard cells, trichomes, germinating pollen grains and tubes, root tips of embryos and lateral roots, seed coat and stipules; suggesting a role for *A. thaliana* SUC3 in the import of sucrose into sink tissues. This interpretation is supported by the observed induction of SUC3 expression upon wounding, since wounded cells/tissues behave as a sink cell/tissue. Further analysis showed that *A. thaliana* SUC3 – promoter:::GUS plant reproducibly gave a very strong GUS staining at all sites where leaves or stem sections were cut before incubation with staining solution. This suggests that wounding enhances the activity of SUC3 and that it is most likely responsible for the import of sucrose into these wounded tissues. In addition, SUC3 may be involved in the retrieval of sucrose along the transport phloem. It was therefore proposed that the function of the stress induced SUC3 may be the import of carbohydrate from the apoplast after wounding or infection, which is important for the enhanced metabolic needs of the wounded tissues, or it may reflect a mechanism for the efficient reduction of the extracellular carbohydrates which otherwise might be used by pathogens. Also in this study, strong SUC3 – promoter – driven GFP fluorescence was found in callus. Callus is a sink tissue typically formed at the site of wounding. The activity of SUC3 at this site is not unexpected for a gene that is expressed in developmental and environmental induced sinks.

Recently, Kempema et al. (2007) reported the up-regulation of SUC1 in *A. thaliana* after silver leaf white fly (SLWF) nymphs feeding. During the infestation a general down-regulation of photosynthesis genes was observed, but nitrogen and carbohydrate metabolism and transport genes were up-regulated, among which was SUC1. This up-regulation might be given to the fact that insects are themselves an additional nutrient sink to which most of the plant sap is diverted rather than to the sink cells.

In summary the differential regulation of these sucrose transporters infer their characteristic different transport function which is enhanced or repressed in relation to plant physiological state, cellular metabolic status and energy requirements.

Plants contain numerous transporters which allow them to express a coordinated developmental pattern that participates in various stress responses. Immunolocali-zation studies of sucrose transporters have been restricted to only a few plant species and families; including *Arabidopsis*, *solanaceae* and *plantago*. It will be a major challenge in the next few years to decipher the exact role, localization and expression patterns of all en-

coded sucrose transporter proteins present in the *Arabidopsis* genome, and most importantly in agricultural crops during stress conditions such as wounding which plants are often exposed to.

Furthermore, data on monocotyledon sucrose transporters are scarce. Monocotyledon seeds of rice, maize, wheat, barley, oat, sorghum, millet are of great agronomic significance, since they represent the main source of carbohydrates for humans. Understanding of sucrose transporter expression and regulatory activities involved in distribution of post-phloem photoassimilates and accumulation of carbohydrates appear to be of major economic importance, most importantly during insect herbivory or pathogen attack as a result of wounding. This will help in understanding the individual roles of each sucrose transporter in plant stress physiology. This will also further contribute more information to the poorly understood physiological processes such as: pollen/seed development, root and shoot growth, stomata opening, plant-pathogen interaction and resistance signaling mechanisms during plant wounding. In future this information may aid in breeding programmes for the development of hybrid varieties that can withstand biotic stress conditions such as insect herbivory or pathogen attack as a result of wounding. Studies should however be conducted to unravel how plants distinguish and respond to the different threats posed by mechanical injury and herbivore attack.

Several classes of sucrose transporters are still unknown in several plant species, so are the sucrose transporters that mediate the efflux of sucrose across the tonoplast and the plasma membrane. There are the possibilities of sucrose efflux carriers that supply sucrose to injured cells in addition to the normal plasma membrane sucrose transporters, but this still has to be investigated. To-date no sucrose efflux carrier has been isolated.

The study of sucrose transporters in relation to different stress physiologies should become a rapidly evolving area. Major attention should be focused on the elucidation of the molecular mechanisms that link the effect of diverse stress stimuli most especially wounding to source-sink sucrose regulation. Identifying the promoter regions that regulate sucrose transporters will be very important in understanding the information that triggers each group of sucrose transporters to be transcribed and then translated into a functional protein.

More precise information regarding the nature of the signals that trigger different sucrose transporter responses to wounding will also need to be investigated in future.

ACKNOWLEDGEMENT

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